



## Seeds

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# Morphometry of chincuya seeds (*Annona purpurea* Moc. & Sessé ex Dunal) and embryonic growth under dry warm storage

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**Abstract.** In Mexico, *Annona purpurea* Moc. & Sessé ex Dunal, chincuya is found in mountains and family gardens. It is used as food (fruits), traditional medicine (leaves, stems, roots, seeds, bark), wood in construction, papermaking, carpentry, rope making, and as fuel. There is not enough information on the seed, nor on germination management, which is scarce, prolonged and erratic. Considering that the morphophysiological dormancy is the probable cause, it was necessary, within a broader project, to start with the characterization of the seed and determine the effect of dry warm storage (DWS) on it. The perimeter and length of the embryos increased due to the increase in length of the cotyledons and the hypocotil root axis, while the middle hypocotyl area decreased, forming an acinturated embryo. These data indicate that the embryos of chincuya are underdeveloped since they grew and modified their shape, due to the effect of DWS, until the 6th month. This development of the embryo within the seed during dry warm storage confirms the presence of morphological dormancy. This is the first report of embryo growth and characterization of chincuya seeds.

**Keywords:** morphological dormancy, underdeveloped embryos, post-maturation.

## Morfometria de sementes de Chincuya (*Annona purpurea* Moc. & Sessé ex Dunal) e crescimento embrionário sob armazenamentos quente e seco

**Resumo.** No México, *Annona purpurea* Moc. & Sessé ex Dunal, a chincuya é encontrada em montanhas e em pomares domésticos. É utilizada como alimento (frutas), na medicina tradicional (folhas, caules, raízes, sementes, casca), como madeira na construção, na fabricação de papel e de cordas, carpintaria e como combustível. Não há informações suficientes sobre a semente, nem sobre o manejo da germinação, que é reduzida, prolongada e errática. Considerando que a dormência morfo-fisiológica seja a provável causa da dormência, considerou-se necessário, dentro de um projeto mais amplo, começar pela caracterização das sementes e pela determinação do efeito do armazenamento a seco e a quente sobre elas. O perímetro e o comprimento dos embriões aumentaram durante o armazenamento devido ao aumento do comprimento dos cotilédones e do eixo do hipocótilo radicular, enquanto a área do hipocótilo médio diminuiu, formando um embrião em forma de 'cintura'. Esses dados indicam que os embriões de chincuya são subdesenvolvidos, uma vez que crescem e mudam de forma durante os armazenamentos seco e quente, até ao sexto mês. Este desenvolvimento do embrião dentro da semente, durante o armazenamento, confirma a presença de dormência morfológica. Este é o primeiro relato de crescimento embrionário e da caracterização de sementes de chincuya em armazenamento.

**Termos de indexação:** latência morfológica, embriões subdesenvolvidos, pós-maturação.

### Introduction

The Annonaceae family is one of the most primitive within the angiosperms, and is found in tropical and subtropical areas, conditioning its germination behavior. Chincuya seeds do not germinate uniformly, they require prolonged periods (more than 20 days) and usually germination is less than 30% (VIDAL-LEZAMA et al., 2015, 2019; FERREIRA et al., 2016). Seedling establishment depends on germination and occurs in physiologically mature seeds without dormancy; understanding germination physiology, starting with the anatomical and morphological characteristics of the seed, will allow the creation of better strategies for germplasm management. Taiz and Zeiger (2006), indicate that during embryogenesis some events occur, by which the basic architecture of the plant is established, including the construction of basic forms (morphogenesis), association between them as organized and functional structures (organogenesis), as well as the differentiation of cells that form tissues (histogenesis). According to Sano et al. (2015) and Costa et al. (2017), the seed has

reached maturity if it has completed its morphological and physiological development and coincides with the cessation of dry matter accumulation. During germination, the intense metabolic activity, is initially manifested with the growth of the root meristem. The establishment of the seedling depends on the correct sequence of the germination phases, and it only occurs in those mature seeds that have not entered dormancy or have overcome it. The primary dormancy of the seed develops before it separates from the parent plant and is established during maturation. Secondary dormancy is defined as that which is acquired after the seed has been disseminated or harvested (FINCH-SAVAGE; LEUBNER-METZGER, 2006). Once the seed has matured, it may or not enter the dormant state, a term that defines a complex network of evolutionary adaptation strategies, resulting from the coexistence of the species with the environmental factors that have surrounded it. Carvalho and Nakagawa (2000) identified the existence of physical, morphological, physiological, morpho-physiological and combined (physi-

cal plus physiological) dormancy. Baskin and Baskin (2014) noted that, if the embryo has radicle, cotyledons and a relatively small or absent amount of endosperm, it is differentiated. Therefore, morphological dormancy is observed in seeds with undifferentiated and subsequently differentiated embryos, but they remain underdeveloped, or they are differentiated but underdeveloped embryos and, in both cases, they should grow before radicle emergence. Sautu et al. (2007) found that *Annona spraguei*, *Xylopia aromatica* and *X. frutescens* have morpho-physiological dormancy. Baskin and Baskin (2005) also report that many tropical rainforest species are dormant. Seeds with physiological dormancy are permeable to water, but the embryo has a physiological impairment resulting in poor growth potential; as dormancy release occurs, the potential increases until germination is possible (BASKIN; BASKIN, 2014). Warm storage (also called post-ripening) has been used to release dormancy in seeds of *Bromus tectorum*, *Arabidopsis thaliana* and *Nicotiana tabacum* (FINCH-SAVAGE; LEUBNER- METZGER, 2006) and in *Fraxinus excelsior* it was associated with a decrease in abscisic acid content. Taking into account the lack of information on seed and especially on seed conservation and germination management, which is scarce, prolonged and erratic, and the hypothesis that morpho-physiological dormancy is the cause, it was considered necessary, as part of a broader project, to begin with seed characterization and determine the effect of dry warm storage (DWS).

## Materials and Methods

Morphometric analysis was carried out on seeds extracted, washed and dried for two days at room temperature, from mature fruits recently collected from the locality of Las Salinas, Municipio, Chicomuselo, Chiapas, Mexico. We evaluated five treatments: 0, 3, 6, 6, 9 and 12 months of DWS, where the seed was kept in a closed plastic bag and in darkness in an oven at  $25 \pm 3$  °C constantly. The embryos were obtained by soaking the seeds without seed coat in water

for 12 h and cut longitudinally with a scalpel; when detached from the endosperm, the embryos were collected with a brush and placed on slides with water, to be observed under a photo microscope (III Carl Zeiss®) and 10X objective and photographed with a digital camera (PaxCam 3®). Considering the large size of the whole seeds, photographs of the seeds and seed coatings were taken by scanning them (HP Scanjet 4070®) and subsequently processed with ImageJ software (V. 1.51). Seed description was analyzed with descriptive statistics and an ANOVA in a completely randomized design with four replications of 20 seeds, the statistical significance was observed, and Tukey's multiple mean comparison was applied with 95 % reliability.

## Results and Discussion

### Morphometric description

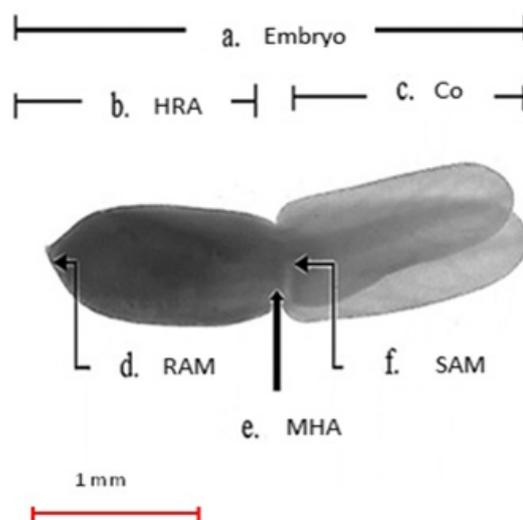
The external appearance of the chincuya (*Annona purpurea* Moc. & Sessé ex Dunal) seed shows an opaque dark brown color, with obovate shape (Figure 1, a, e), with sharp and semilignified tip, this one of lighter tone than the rest; the cover (Figure 1, c) that firmly wraps the endosperm is woody, hard, fibrous, of rough and striated texture; when dry, a very thin and whitish cuticle can be distinguished on it, traces of the sarco testa that wraps each seed (Figure 1, e). The average fresh weight of the whole seed was  $1.56 \pm 0.126$  g, 2.64 cm long and 1.35 cm wide (Table 1). The endosperm (Figure 1, b) is creamy white to yellowish, which completely wraps the embryo (Figure 1, d), it is very hard and as it loses moisture, measuring on average 1.91 cm long and 0.95 cm wide (Table 1), it is ellipsoidal with numerous folds covered by a thin brown papyraceous testa (Figure 1, c). In cross section, the ruminations are spiniform as has been defined in other species of the same family (VAN SETTEN y KOEK-NOORMAN, 1992). When the seed cover is free, we can appreciate the micropyle in the middle of the elliptical and sunken hilum, as well as the pericalaza, both of brown color (Figure 1, b), this last one is a distinctive structure of the Annonaceae



**Figure 1.** Seeds of chincuya and components. a. Moist with dark brown seed coat. b. Without seed coat, showing ruminated endosperm (End), sunken hilum (Hi), micropyle (Mi) and brown pericalaza (Pe). c. Open seed coat. Light brown laminated ruminations. Different thickness in the upper part compared to the sides and base. d. Small, soaked and differentiated embryo, observed under light and objective microscope at 10X. e. Dry seeds with seed coat and traces of sarco testa. f. Micropylar plug with elongated protuberance in the upper part.

family (SVOMA, 1997). The micropylar plug (Figure 1, f) is located in the hilar zone blocking the micropylar, measuring between 1.77 to 4.50<sup>2</sup> in area, on average  $3.08 \pm 0.55 \text{ mm}^2$  and median of  $3.12 \text{ mm}^2$ , is a woody, porous, cone-shaped structure with a long filament (0.47 mm) superiorly located in the micropylar canal and two long extensions visible in the mid-plane. The embryo (Figure 1, d and 2, a) is straight, small on average 3.4 mm long, 0.58 cm wide (MHA) and  $2.58 \text{ mm}^2$  of area (Table 2) white matte, located near the hilum and centered on the endosperm, with the radicle pointing toward the micropyle. Two very thin foliaceous cotyledons are noted (Figure 2, c), measuring 1.71 mm long and 0.79 mm wide (Table 2). Furthermore, the following embryo components were included: root hypocotyl axis (Figure 2, b) measuring 1.68 mm long and 0.78 mm wide (Table 2) and the middle hypocotyl area (Figure 2, e) dividing the embryo in half, between the cotyledons and the root hypocotyl axis, measuring 0.58 mm wide (Table 2). Table 1 shows that chincuya seed is large and heavy compared to the seeds of other anonas, for

example *A. cherimola* (1.5- 2 cm), *A. muricata* (0.37 g), *A. reticulata*, *A. squamosa* (1.5 – 2 cm and 0.25 g) according to Manica et al. (2003).



**Figure 2.** Chincuya soaked embryo. Observed under light microscope and 10X objective. a. Whole embryo. b. HRA. Hypocotyl radicular axis. c. Co. Cotyledons. d. MAR. Root apical meristem. e. RMHR. Middel hypocotyl area. f. MAC. Shoot apical meristem.

Table 1. Whole seed dimensions, seed coat thickness and endosperm of chincuya seeds.

		Mi-ma <sup>z</sup>	Average	Median	CV <sup>y</sup>
WHOLE SEED (cm)	Length	1.49 – 4.33	2.64 ± 0.40	2.6	15.4
	Width	0.73 – 2.13	1.35 ± 0.25	1.3	19.0
	Perimeter	5.52 – 15.71	8.33 ± 1.69	8.0	20.4
	Area (cm <sup>2</sup> )	1.34 – 5.29	2.78 ± 0.75	2.6	26.9
SEED THICKNESS COAT (mm)	Middel	0.07 – 0.19	0.12 ± 0.02	0.1	20.7
	Tip	0.26 – 0.65	0.47 ± 0.07	0.5	15.3
	Base	0.05 – 0.20	0.12 ± 0.02	0.1	19.4
ENDOSPERM (cm)	Length	1.55 – 2.19	1.91 ± 0.15	1.9	7.9
	Width	0.67 – 1.18	0.95 ± 0.10	0.9	10.7
	Perimeter	4.27 – 9.79	5.19 ± 0.57	5.2	11.1
	Area (cm <sup>2</sup> )	0.051 – 1.98	1.49 ± 0.23	1.49	15.8

<sup>z</sup> Minimum-maximum values. <sup>y</sup> Percentage coefficient of variation.

Number of observations=350 whole seeds, 100 seed coats and endosperms.

Table 2. Dimensions of soaked whole embryos, cotyledons, middle hypocotyl area and hypocotyl root axis of chincuya seeds.

		Mi-ma <sup>z</sup>	Average	Median	CV <sup>y</sup>
EMBRYO (mm)	Perimeter	6.25-19.14	9.12±1.29	8.96	14.22
	Length	2.44-4.12	3.40± 0.29	3.41	8.61
	Length MHA <sup>x</sup>	0.34-1.01	0.58±0.10	0.58	18.26
	Area (mm <sup>2</sup> )	1.44 – 4.11	2.58 ± 0.43	2.55	16.69
COTYLEDONES (mm)	Length	1.09-2.06	1.71±0.18	1.74	10.73
	Width	0.46-1.11	0.79±0.11	0.79	13.51
HYPOCOTYL ROOT AXIS (mm)	Length	0.82-2.17	1.68±0.19	1.68	11.52
	Width	0.47-1.10	0.78±0.10	0.79	13.04

<sup>z</sup> Minimum-maximum values. <sup>y</sup> Percentage coefficient of variation. <sup>x</sup> Middle hypocotyl area

Number of observations = 270

The value estimated for the ratio between the embryo and the complete seed was 0.128, while for the endosperm it was 0.178; both values show how small the embryo is. According to Forbis et al. (2002), the values of this ratio increase according to the location of the species in the phylogenetic tree, and they indicate that in mature seeds of primitive angiosperms, the embryo is small and is soaked in abundant endosperm, characteristics shared by the seeds of chincuya. This confirms that it is a species of a basal family in phylogenetic terms (CHATROU, 1999) and would explain the morphophysiological dormancy, as a plesiomorphic character (SAUTU et al., 2007). Table 1 shows the variation in seed size, which in the maximum case can measure up to 4.3 cm in length and more than 5 cm<sup>2</sup> in area; these dimensions are among the highest reported for the ge-

nus *Annona* except for *Anonidium mannii*, which is between 3.5 and 4.8 cm long (VAN SETTEN; KOEK-NOORMAN, 1992). Vandeloock and Van Assche (2008), observed that the embryo to seed (E:S) ratio went from 0.14 to 0.92, when comparing freshly collected seeds of *Sanicula europea* and seeds buried for more than 4 months. In *A. purpurea* the E:S ratio values went from 0.125 at the time of harvesting to 0.134 after 6 months of DWS, evidencing embryonic growth (Tables 1 and 3). The seed coat has a similar thickness in the middle and basal part (Table 1) however, in the upper part, it is almost 4 times thicker than the base. The seed coat, although rigid, lignified and thick, it does not interfere with imbibition because it is permeable (VIDAL-LEZAMA et al., (2008); FERREIRA et al., (2014, 2016), also in other species of the genus *Annona*, the non-existence of

physical dormancy is demonstrated by imbibition of water and growth regulators in several experiments, as has been documented by Marroquín–Andrade et al. (1997) and Ferreira et al. (2014, 2016) in ilama (*A. macrophyllata* sinonimia de *A. diversifolia*), by Vidal-Lezama et al. (2011) in saramuyo (*A. squamosa*), by Vidal-Lezama et al. (2006) in chirimoya (*A. cherimola*), by Da Silva et al. (2007) in *A. crassiflora*, in soursop (*A. muricata*) by Vidal-Lezama et al. (2017) while in atemoya (hybrid *Annona X atemoya* Mabb) and *A. emarginata* reviewed by Ferreira et al. (2019).

Table 1 shows that the endosperm is long and wide in relation to the seed coat while area and perimeter of whole seeds have a wide range of variation, however, the averages and medians are very similar. The length:width ratio is almost 2:1, so its shape is defined as ellipsoid. The thickness of the tip of the seed coat (Table 1), equals the length of the filament of the micropylar plug (Figure 1, f).

The records indicate that the area of the micropylar plug ranged from 1.779 to 4.501 mm<sup>2</sup>, on average  $3.08 \pm 0.55$  mm<sup>2</sup> and median of 3.123 (coefficient of variation of 17.82%). The dimensions of the embryos are shown in Table 2 as with whole seeds, the values of the embryo components coincide in average and median, except for perimeter. It is observed that these are tiny embryos, four times longer than wide. The almost perfect symmetry is remarkable since the lengths of cotyledons and root hypocotyl axis are almost equal. According to the criteria of Martin (1946), small embryos occupy less than 25% of the total volume of the endosperm, the embryo of chincuya seeds occupies only 17%. Observations of embryos with light microscopy clearly identified the root hypocotyl axis (Figure 2, b) and cotyledons (Figure 2, c) in agreement with Baskin and Baskin's (2014) definition of fully developed embryos.

When soaked, the cotyledons are separated, and it is possible to appreciate the venation. Our results indicate that the embryos of

chincuya seeds are small but differentiated, since the shape is not globular, heart-shaped or torpedo-shaped, which are forms prior to that of a differentiated embryo, as dictated by embryogenic studies of dicotyledons (RUDALL, 2007).

### Effect of dry warm storage.

Effect of warm dry storage. Embryo growth under DWS was evident until the sixth month (Tables 3 and 4). Similar results were observed by Han et al. (2010), in seeds of *Michelia yunnanensis* with embryos that share with *A. purpurea*, class (Magnoliopsida) and taxonomic order (Magnoliales), and the large amount of endosperm and how it encloses it; they also coincide in the description of small embryos, with differentiated root and cotyledons, E:S ratio at the time of dispersal of 0.15, the same authors indicating that the seeds are mature with underdeveloped embryos, so called because they observed that the embryos grew inside the seed, when cold stratification was applied for 60 days. The data in Table 3 revealed consistent increases up to the sixth month in embryo perimeter and length, while embryo area and MHA width decreased. The DWS favored the growth of chincuya embryos up to six months, subsequently the values of both the area and length of the whole embryo decreased (Table 3), as well as the length and area of the cotyledons and the area of the hypocotyl axis (Table 4), probably as a manifestation of damage due to prolonged storage, because of the high content of fats contained (VIDAL-LEZAMA et al., 2019). Table 3 shows a different formation of the embryo with time of storage. From an elongated vertical shape, it changed to a longer and narrower vertical shape. The reduction in MHA could also be observed with time. At the time of extraction, the embryos were larger in MHA and with time it decreased, forming an "inked" embryo. This morphological change is a further indicator of the occurrence of embryo development within the seed before root emergence coinciding with the definition of morphological dormancy of Baskin

**Table 3.** Average perimeter, area, length and width of soaked embryos of chincuya seeds, under the effect of dry warm storage intervals.

MONTHS STORAGE	PERIMETER (mm)	AREA (mm <sup>2</sup> )	LENGTH (mm)	WIDTH MHA <sup>x</sup> (mm)
0	8.332 ± 0.20 C <sup>y</sup>	2.865 ± 0.07 A	3.306 ± 0.04 B	0.753 ± 0.01 A
3	9.630 ± 0.14 A	2.553 ± 0.05 B	3.344 ± 0.03 B	0.592 ± 0.01 B
6	10.368 ± 0.28 A	2.615 ± 0.09 AB	3.540 ± 0.06 A	0.542 ± 0.02 B
9	8.862 ± 0.13 C	2.605 ± 0.04 B	3.428 ± 0.03 AB	0.547 ± 0.00 B
12	9.032 ± 0.13 BC	2.436 ± 0.04 B	3.453 ± 0.03 AB	0.560 ± 0.00 B
LSD <sup>z</sup>	0.741	0.256	0.176	0.052

<sup>z</sup>LSD. Least significant difference. <sup>y</sup>Means with the same letter within columns, are not significantly different ( $\alpha = 0.05$ ), according to Tukey. <sup>x</sup>Middle hypocotyl area.

**Table 4.** Average cotyledon and root hypocotyl axis of soaked embryos of chincuya seeds, under the effect of dry warm storage intervals.

MONTHS STORAGE	COTYLEDONES (mm)		ROOT HYPOCOTYL AXIS (mm)	
	Length	Width	Length	Width
0	1.638 ± 0.03 B <sup>y</sup>	0.868 ± 0.01 A	1.583 ± 0.03 B	0.888 ± 0.01 A
3	1.680 ± 0.02 AB	0.829 ± 0.01 B	1.666 ± 0.02 B	0.771 ± 0.01 BC
6	1.782 ± 0.04 A	0.771 ± 0.02 BC	1.756 ± 0.04 A	0.821 ± 0.02 B
9	1.715 ± 0.01 AB	0.784 ± 0.10 BC	1.707 ± 0.01 A	0.783 ± 0.00 BC
12	1.748 ± 0.02 A	0.759 ± 0.01 C	1.712 ± 0.02 A	0.755 ± 0.01 C
LSD <sup>z</sup>	0.109	0.062	0.115	0.057

<sup>z</sup> LSD. Least significant difference. <sup>y</sup>Means with the same letter within columns, are not significantly different ( $\alpha = 0.05$ ), according to Tukey

and Baskin (2014). The modification in the formation of the embryo may be the result of growth at the poles of the embryo, where the root and shoot apical meristems are located, because in the MHA, the shoot apical meristem is located, which is activated by the effect of the DWS and in preparation for the next germination. Chincuya embryos at the time of seed dispersal are small and increase in size after 6 months of DWS but remain small compared to the endosperm. Finch-Savage and Leubner-Metzger (2006) and Baskin and Baskin (2014), agree that seeds with morphological dormancy have underdeveloped but differentiated embryos that should grow before root emergence, this is the case of chincuya. The growth of embryos could be due to the presence of the relationship between growth promoters and abscisic acid, in favor of the former (FIGUEIREIDO et al., 2016) and/or the combination of the effect of seed moisture content with the temperature of 25 °C, because, as mentioned by Smith et al. (2010), temperatures between 25 and 30 °C are

appropriate for maximum germination for most tropical tree seeds. Baskin and Baskin (2014) suggested that the environmental conditions required for embryo growth may be similar to those needed for germination. Another possible explanation is that the embryos never stopped growing and never entered the seed dehydration phase, as occurs in orthodox seeds. Smith et al. (2010), indicated that, due to the high moisture content of tropical seeds at the time of extraction, embryo growth and germination proceed without interruption. Duke (1969) mentioned that some seeds appear to have no dormant period (*Annona*, *Durio*, *Myristica* and *Thalassia*) and that after morphological and physiological maturation, the seed may delay readiness for germination until after dispersal. The seeds of primitive gymnosperms and some angiosperms lack dormancy, and since there is a prolonged process after maturation, the absence of dormancy seems to be a primitive character. In the case of chincuya seeds, more extensive and integrative studies are needed to de-

fine if it is only about changes in size and shape or if there are also metabolic modifications, which would help to define if it also has physiological dormancy, besides morphological dormancy. There is a possibility that the chincuya seeds, when separated from the parent plant and the fruit, have a reduced supply of nutrients and water and therefore possibly do not continue to grow at the same rate as they did before falling from the parent plant and perhaps were never quiescent (primary dormancy). To test this hypothesis, the collection of the chincuya fruits could be delayed, as well as the extraction of the seeds from the fruit, thus favoring the growth of the embryos, while the flow of substances that feed the seeds probably continues to function and there-

fore the seeds achieve a more developed embryo.

## Conclusions

Dry warm storage of seeds favored embryo growth. These data indicate that chincuya (*Annona purpurea* Moc. & Sessé ex Dunal) embryos are underdeveloped because they grew and modified their shape under dry warm storage, until the sixth month. This development of the embryo inside the seed before root emergence confirms the presence of morphological dormancy. Based on the results of the present study, future studies should explore other types of dormancies, and germination studies will be essential for this purpose.

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