

## WATER UPTAKE BY *Annona diversifolia* SAFF. AND *A. purpurea* MOC. & SESSÉ EX DUNAL SEEDS (ANNONACEAE)<sup>1</sup>

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**ABSTRACT** - Annonaceae seeds are known by presenting dormancy mechanisms, whose reports ranging from coating impermeable to the physiological dormancy. By this way, the present study aimed to evaluate water uptake in *Annona diversifolia* Saff and *Annona purpurea* Moc & Sessé ex Dunal seeds. For this study, seeds were placed under immersion in distilled water, and used four replicates of 25 seeds of each species, which were weighed during the 480 hours that were immersed. To determine the place of purchase of water, *Annona diversifolia* seeds were sealed with paraffin at different locations. Based on the results, seeds from both species reached the phases I and II of water uptake, which indicates they are not hard; however, germination (Phase III) was not reached. *Annona diversifolia* seeds completed Phase I with, 50h and *Annona purpurea* with 70h from imbibitions begin, which shows that even slowly, water is acquire.

**Index terms:** Annonaceae, propagation, seed dormancy, imbibition.

### AQUISIÇÃO DE ÁGUA EM SEMENTES DE *Annona diversifolia* SAFF. E *A. purpurea* MOC. & SESSÉ EX DUNAL (ANNONACEAE)

**RESUMO** - As sementes de espécies da família Annonaceae são conhecidas por apresentarem mecanismos de dormência, cujos relatos vão desde a impermeabilidade do tegumento até a dormência fisiológica. Neste sentido, o objetivo do presente trabalho foi avaliar a impermeabilidade à água das sementes de *Annona diversifolia* Saff e *Annona purpurea* Moc & Sessé ex Dunal a partir do estudo da curva de aquisição de água. Para a realização do estudo, sementes foram colocadas sob imersão em água destilada, sendo utilizadas 4 repetições de 25 sementes de cada espécie, as quais foram pesadas durante as 480 horas em que ficaram imersas. Para determinar o local de aquisição de água, sementes de *Annona diversifolia* foram vedadas em diferentes locais com parafina. Com base nos resultados, pode-se afirmar que sementes de ambas as espécies apresentam Fase I e II da germinação, o que indica que as sementes não são impermeáveis; contudo, a Fase III não é alcançada. As sementes de *Annona diversifolia* completam a Fase I com 50 horas, e as de *Annona purpurea*, com 70 horas do início da embebição, o que demonstra que, mesmo de maneira lenta, a água é adquirida.

**Termos para indexação:** Anonáceas, propagação, dormência de sementes, embebição.

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## INTRODUCTION

To meet the market demand to fruits of the Annonaceae family are necessary areas of high productivity, which begins with the propagation of seedlings, derived from productive clones. The propagation method most used in Annonaceae is grafting, in which one rootstock is formed from seeds (BARON et al., 2013; LEMOS et al., 2013). Thus, it is a constant search for alternative species such as *Annona diversifolia* Saff and *Annona purpurea* Moc & Sessé ex Dunal, that are potential species to be used in the expansion of production areas as rootstock to other Annonaceous, so as for *in natura* consumption, food industry or to discoveries of new molecules for pharmaceutical industry (LUNA-CAZARES et al., 2006; LOBO et al., 2007; DE-LA-CRUZ et al., 2012). However, Annonaceae seeds are known to have different types of dormancy. There are reports of morphophysiological dormancy (BASKIN Y BASKIN, 1998), presence of rudimentary embryo (SMET et al., 1999) and problems of impermeability teguments (SVOMA, 1998; SMET et al., 1999).

Some studies to overcome seed dormancy with the application of plant growth regulators are been done in Annonaceae (SMET et al., 1999; FERREIRA et al., 2002; LIMA-BRITO et al., 2006; SILVA et al., 2007; BRAGA et al., 2010; OLIVEIRA et al., 2010; COSTA et al., 2011; CORSATO et al., 2012) and in this cases are necessary to determine the time of immersion treatments in the solutions, which is made from the elaboration of the acquisition water curve. Furthermore, obstructions on purchase of water by seeds are also detected with the completion of this curve, as well as times of osmotic conditioning (VARIER et al., 2010).

During the acquisition of water by seeds, metabolic processes are activated, in order to provide energy for the resumption of embryo development (BEWLEY et al., 2013). The beginning of water input by seeds, called Phase I of germination or soaking is characterized by rapid acquisition of water, as a result of a high water potential gradient between the seed and the environment, mainly caused by the low matric potential seed (HILHOST, 2007). With the increased free water availability and metabolism in the seed, the matric potential leaves to be responsible for the water entry into the seed, which indicates entry into Phase II of the curve, *lag* phase. In Phase II, therefore, is when all matrices reach full hydration, the matric potential becomes zero and the osmotic potential becomes responsible for the movement of water into the seed (BEWLEY et al., 2013).

During Phases I and II of the acquisition of water, enzymes are synthesized and activated in response to hormonal activity (GUBLER et al., 2005). Thus, the entry of water in quiescent seeds occurs together with *the new* synthesis of hormones, such as gibberellins, and enzymes like  $\alpha$ -amylase, which act on the storage material degradation (KUCERA et al., 2005; YAMAGUCHI, 2008; TAIZ; ZEIGER, 2013). According to Nonogaky et al. (2010) is during this phase that the genetic regulators of germination act, either to promote or inhibit entry into Phase III, whereas seeds that don't produce gibberellins do not complete germination. Thus, seeds with physiological or morphophysiological dormancy can sustain a low level of metabolic activity and Phase II can be prolonged for months or years (HILHORST, 2007).

In this context becomes evident the importance of understanding the acquisition of water phases to facilitate the detection of dormancy mechanisms related with the wither control and also in determining the time to pre-germination treatments in solutions with plant growth regulators or osmotic conditioning.

Thus, the hypothesis to be tested in this experiment is that seeds of *Annona diversifolia* and *A. purpurea* are not hard (water impermeable), perform Phases I and II of germination, but even with the purchase of water and considering that seeds are dormant, do not complete germination (Phase III).

## MATERIAL AND METHODS

Fruits of *Annona diversifolia* Saff. (papaua) and *Annona purpurea* Moc & Sessé ex Dunal (chincuya) were obtained in home orchards, and in local markets in the city of Tuxtla Gutiérrez, Chiapas, Mexico. The seeds were manually extracted under running water and immersed in a solution of sodium hypochlorite, 10% (i.a.) for 5 minutes, rinsed with distilled water and kept on filter paper in a laboratory bench at 25 °C (+2 °C) for seven days to surface drying.

The water content of the seeds was determined employing four replications of 25 seeds, by the method of drying at 105 °C for 24 hours (BRASIL, 2009). The tetrazolium test to assess the viability of the seeds was performed in four replicates of 25 seeds at the beginning and end of the test. The treatments consisted of the times in which the seeds remained immersed in distilled water, in order to determine the three phases of the acquisition water curve. After determining the initial moisture content of the seed lot, four replicates of 25 seeds were placed in

beckers containing distilled water with aeration and maintained in an incubator brand CONVIRON® at 30 °C (± 2 °C), relative humidity 50 to 60% in the absence of light.

The weight measurements were taken every hour, for eight hours (h), and after 8 h were performed at 24 h and 32 h. The weight continued every 8 h until reaching 120 hours of immersion and, after, every 24 h to 480 h of immersion. In each time, the seeds were removed from the containers, weighed and returned. The water was replaced in each evaluation and maintained oxygenation with aquarium pumps. With the weighing data were calculated water content of the seeds over time, considering the moisture content obtained before immersion of the seeds in the treatment (Time = 0), after four hours of soaking, and so forth, up to 480 h of immersion. The results are shown in moisture content (%) in relation to the initial mass of dry matter (BRASIL, 2009).

To study the variation in water content was adjusted logistic equation model  $y = a / [1 + \exp(b \cdot c \cdot x)]$ , with  $y$  = moisture content (%),  $x$  = time (in hours). To calculate the change in Phase I to Phase II of water acquisition was used the calculation of asymptotic point of deceleration (PDA), as proposed by Mischan et al. (2011).

To determine the location where the water is acquired it was used *A. diversifolia* seeds as model. They were divided into five groups of 100 seeds, each were sealed (obturated) with paraffin as follows: uncovered seed; total obturated seeds, uncovered seed coat (seeds with the hilum obturated); seeds with hilum and suture (rafe-antirafe) obturated; seeds only with hilum uncovered. All groups were placed in distilled water (pH 6.5) at 30 °C and relative humidity of 50 to 60% in the absence of light within a germinator CONVIRON® and were weighed for periods of 0, 1, 2, 3, 4, 7, 23, 47, 56, 72, 80 and 96 hours. Results are expressed as the average of three repetitions in percentage water gained per gram of seed.

## RESULTS AND DISCUSSION

From the calculations proposed by Mischan et al. (2011) was possible to establish the end of Phase I of water acquisition, with determining the asymptotic point of deceleration. This point indicates the approximate value, when there is a reduction in acquisition rate of water by the seed, indicating that the matric potential approaches zero, resulting in the stability of water acquisition by *Annona diversifolia* Saff. and *A. purpurea* Moc & Sessé ex Dunal (Figures 1 and 2 respectively) and that entered into Phase II

seed germination.

Phase I of the water acquisition (imbibition) lasted approximately 50 hours in seeds of *A. diversifolia* and seeds which had an initial moisture content of 9.04% (before the start of imbibition) reached 31.16% water content at the end of this phase. The same pattern of response was observed in seeds of *A. purpurea*, which had an initial moisture content of 16.54%, and reached the stability curve around 70 hours, with a water content of 31.18% (Figures 1 and 2).

The acquisition of water by the seeds occurs due to the difference between the water potential of these and the environment and, bigger the difference, faster the rate of imbibition. In this case, the first acquisition phase of water (Phase I) tends to be rapid and should last for 1 to 2 hours (BEWLEY et al., 2013). To Marcos Filho (2005) Phase I occurs for eight to sixteen hours, reaching moisture content exceeding 45% in cotyledon seeds 30-35% in endospermatic seeds. And yet, according to Carvalho and Nakagawa (2000) between one and two hours seeds complete Phase I, achieving the water content ranging between 35% and 40% for cotyledons seeds and 25% and 30% for endospermatic seeds.

The species in this study showed slowly water acquisition, over that proposed by Bewley et al. (2013) which suggest the duration of one to two hours to the Phase I. It is noted that, while *A. diversifolia* and *A. purpurea* seeds presenting Phase I duration of about 50 and 70 hours, respectively, Ferreira et al. (1997) observed that *A. squamosa* L. seeds stabilized the water uptake with approximately 5 hours of immersion while, at 12 hours, atemoya seeds (*A. cherimola* x *A. squamosa*) continued in Phase I. Subsequently, Ferreira et al. (2006) determined Phase I in atemoya seeds (*A. cherimola* x *A. squamosa*) lasting 30 to 36 hours. Moreover, in a study of *Annona emarginata* seeds, Costa et al. (2011) found that to complete Phase I were required 60 to 72 h of imbibition (Table 1).

It should be noted that, regardless of the seeds moisture content, Phase I was completed, in both species, when water content was achieved of approximately 31%, regardless of the time taken to reach this water content. In this case, *A. diversifolia* imbibes water faster than *A. purpurea*. Thus, it is evident that the change from Phase I into Phase II is characterized by water content reached and not according to the time taken to reach it. Likewise Costa et al. (2011) also obtained 27.85% and 28.35% water content in seeds of *A. emarginata* when completed Phase I (Table 1). These observations are consistent with that proposed by Carvalho and

Nakagawa (2000) since the authors reported that the curve stabilization takes place when the reached moisture content about 25 to 30% and by Marcos Filho (2005) of 35%, in endospermatic seeds. In the case of *Annona* seeds this ratio could be related to the lipid and ruminant characteristic of the endosperm, so the imbibed water could be deposited, in the beginning, between the ruminations caused by the disposal of tegmen.

However, several factors must be considered, such as the resistance by the seed tegument to water entry, the reserves composition, and the environmental conditions at the time of imbibition (BEWLEY et al., 2013).

The acquisition rate of water by Annonaceae seeds may be related to the resistance imposed by the seed tegument, which does not mean that no water is absorbed. As proposed by Svoma (1998) and Smet et al. (1999) Annonaceae seeds have a tegument composed of a woody waterproof cover, indicating that water can penetrate only through the seed hilum, through the micropyle. Another factor that may have resulted in slower acquisition of water by Annonaceae seeds is the largest proportion of lipids in their reservations, about the proteins and sugars (CORSATO et al., 2012). However, contrary to what has been proposed by Svoma (1998) and De Smet et al. (1999) about the impermeability of the covering, the results with wax indicate that at least in *A. diversifolia* there is no impediment to the entry of water into the seed, since water enters through different sites, mainly through the seed coat (testa), the testa suture (rafe-antirafe), and fewer by hilum-micropyle (Figure 3).

With the end of Phase I, lasting 50 h for *A. diversifolia* seeds and 70 h for *A. purpurea* seeds, Phase II was initiated, that was observed up to 480 h from start of immersion.

In Phase II, water acquisition becomes almost zero, because reductions in water potentials of seed are mainly dependent of decreasing in osmotic potential, which in turn, depends on the metabolic activity initiated in Phase I, when the large molecules are degraded lowering the osmotic potential of the environment (BEWLEY et al., 2013). The seeds in this study remained 430 h and 410h (*A. diversifolia* and *A. purpurea*, respectively) on Phase II and did not reach the visible germination, which suggests the presence of some dormancy control. Other important information is that the seeds were alive during the experimental time according tetrazolium test.

When the seeds have physiological or morphophysiological dormancy, the duration of Phase II can be extended (HILHORST, 2007), which

seems to have occurred in this study, since no visible germination was observed (Phase III) after 20 days of assessment. The fact that *A. diversifolia* and *A. purpurea* seeds were alive and did not reach Phase III, indicates the existence of some germination control, as also proposed by Alborno et al. (2006) in relation to *A. purpurea*, and by González-Esquinca et al. (1997) in relation to *A. diversifolia* seeds reporting the need for six to eight months of storage to overcome dormancy.

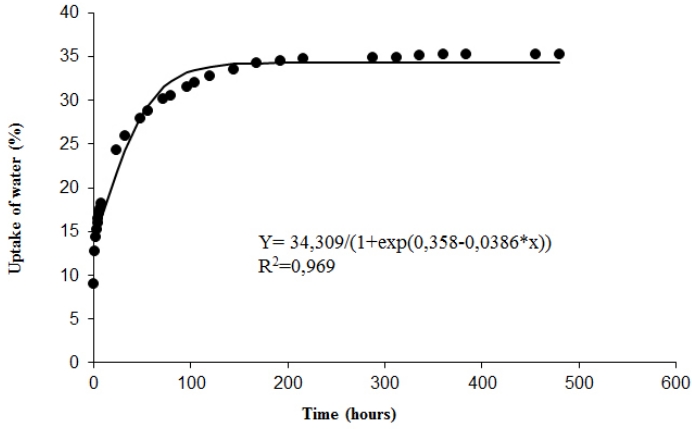


FIGURE 1-Pattern acquisition of water in *Annona diversifolia* Saff. seeds.

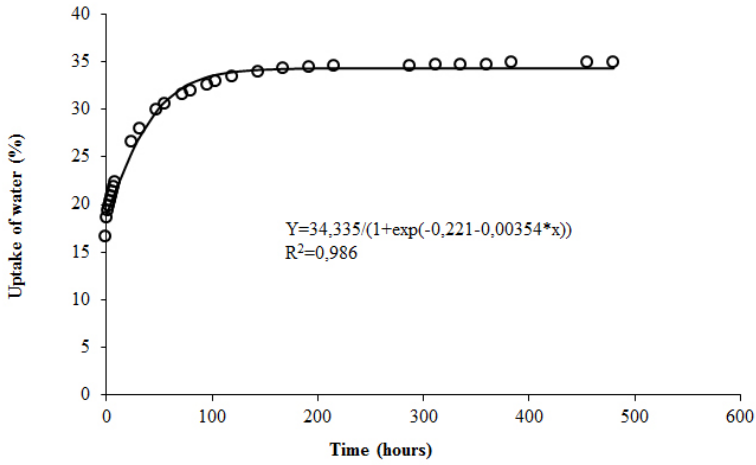


FIGURE 2. Pattern acquisition of water in *A. purpurea* Moc. & Sessé ex Dunal seeds.

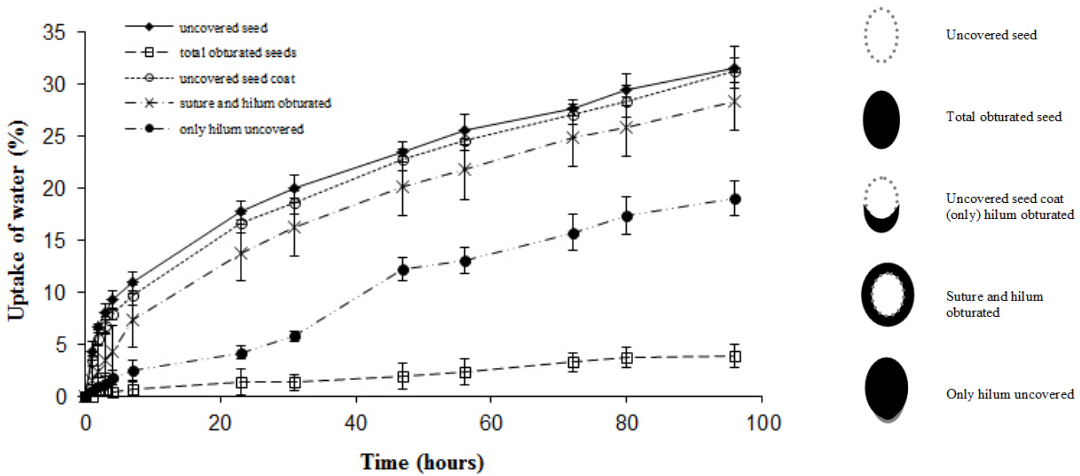


FIGURE 3- Places of water acquisition in *Annona diversifolia* Safford seeds.



**TABLE 1-** Characteristics of pattern acquisition of water in some Annonaceae seeds.

Specie	% Initial water	% Final water	Phase I duration (h) $\psi_m = 0$	Acquisition rate of water (g H <sub>2</sub> O.h <sup>-1</sup> g sem <sup>-1</sup> )	Morfological/Physiological characteristics of seeds	Reference
<i>A. squamosa</i>			92		Permeable seed	Ferreira et al., 1997
<i>A. cherimola</i> x <i>A. squamosa</i>	11	20	27-47	0.19-0.33	Permeable seed, Physiological dormancy	Ferreira et al., 1997 Ferreira et al., 2006 Braga et al., 2010
<i>A. emarginata</i>	10	30.9	60-72	0.27	Permeable seed, tendency to be orthodox, low storage capacity	Iassia et al., 2011 Costa et al., 2011; Corsato et al., 2012
<i>A. diversifolia</i>	9.04	31.16	50	0.4424	Permeable seed, physiological dormancy	Ferreira et al., 2013
<i>A. purpurea</i>	16.54	31.18	70	0.2091	Permeable seed, physiological dormancy	Ferreira et al., 2013

## CONCLUSION

*Annona diversifolia* Saff and *A. purpurea* Moc & Sessé ex Dunal are not impermeable seeds, because they present Phase I and II of germination well characterized, although the acquisition of water takes place slowly and do not reach to Phase III. The slow water acquisition appears to be a characteristic of Annonaceae seeds.

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