

## Propagation of Annonaceous plants

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**Abstract-** This review aims to present advances in studies on the propagation of the Annonaceae species, which includes species of economic importance such as: soursop, custard apple, atemoya and cherimoya. In sexual propagation, advances are mainly related to a better understanding of the stages of seed development, dormancy mechanisms, and germination. In asexual propagation, compatibility studies between grafts and rootstocks are presented, focusing on the expression of genes involved in tissue formation. The cutting method is also discussed, which is another option for the propagation for this group of plants considered difficult to root, approaching endogenous and exogenous factors related to the subject, as well as management strategies that affect the success of this technique.

**Index terms:** Annonaceae, seed dormancy, germination, grafting, cutting.

## Propagação de Anonáceas

**Resumo-** A presente revisão tem por objetivo apresentar os avanços nos estudos sobre a propagação de espécies da família Annonaceae, que inclui espécies de importância econômica como: graviola, fruta-do-conde, atemoia e cherimoia. Na propagação sexuada, os avanços estão relacionados, principalmente, com a melhor compreensão dos estádios de desenvolvimento das sementes, mecanismos de dormência, e germinação , em especial a fase de embebição das mesmas. Na propagação assexuada, são apresentados estudos de compatibilidade entre enxertos e porta-enxertos, com foco na expressão de genes envolvidos com a formação dos tecidos. Também é discutido o método da estaca, outra opção de propagação para este grupo de plantas consideradas de difícil enraizamento, abordando-se fatores endógenos e exógenos relacionados ao tema, bem como as estratégias de manejo que interferem no sucesso desta técnica.

**Termos de indexação:** Annonaceae, dormência, germinação, sementes, enxertia, estaca.

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## Introduction

The Annonaceae family has pantropical distribution and is one of the oldest existing flower groups, with basal lineage estimated to be 112 million years old, with approximately 2100 species and 129 genera (STEVENS, 2016), significantly contributing to the diversity of forests, both for its abundance and its phenological characteristics that allow the survival of organisms that live with it (GOTTESBERGER, 2014; GONZÁLEZ-ESQUINCA et al., 2016). Four species have economic importance, *Annona muricata* L. (soursop), *A. squamosa* L. (custard apple), *A. cherimola* Mill. (cherimoya) and *A. squamosa* x *A. cherimola* hybrid (atemoya), due to their edible fruits and *Cananga odorata*, which produces aromatic substances for perfumery. In addition, the family is recognized as a source of active substances with potent pharmacological and pesticide activities, some of which are expected to be in the near future, new anticancer drugs (LIAW et al., 2016).

The main means of perpetuating species of the Annonaceae family occurs through the seminiferous way, which often presents some dormancy mechanism. In addition, seeds are used for the production of commercial canopy rootstocks, with grafting being the main method of vegetative propagation. Cutting has also been used, but its application has been restricted to the production of seedlings destined to pathogen-free areas in the soil or for the production of rootstocks.

### Seeds: development, germination and dormancy stages.

Although most Annonaceae flowers are self-compatible, protogenic dicogamy acts against self-pollination. Pollination is done predominantly by small beetles (cantarofilia) in diurnal flowers (e.g., *Guatteria*, *Duguetia* and *Annona*) and large beetles (Dynastinae, Scarabaeidae) in nocturnal flowers (e.g., *Annona*, *Duguetia*), but can also be done by thrips, flies, cockroaches and bees (GOTTSBERG, 2014). In *Annona coriacea*, pollination by *Cyclocephala* is more effective than self-pollination and manual cross-pollination (PAULINO-NETO, 2014).

Pollination is followed by coupling, copulation, zygote formation and seed development, which can be divided into three phases, the first characterized by cell divisions, histodifferentiation and morphogenesis of the basic plane of the embryo, as well as high water content (BEWLEY et al., 2013). In *A. squamosa*, the early development of the embryo and endosperm occur after 24h of pollination (SANTOS et al., 2014). In *Annona emarginata*, high cell division was observed, but the embryo was not yet differentiated at 49 days after flowering (DAF), at 91 DAF, the embryo already had differentiated axes and cotyledons and germination

capacity (CORSATO, 2014).

In the second phase of seed development, accumulation of reserves and dry mass in *A. emarginata* occurs between 91 and 116 DAF (CORSATO, 2014). In Annonaceae, the main reserves are lipids, which correspond to 19 to 38% of the dry endosperm mass (RBG Kew, 2016). In *A. squamosa* seeds, 22% of lipids composed of oleic, linoleic, stearic and palmitic acid (47, 23, 13 and 12% respectively) were quantified (RANA, 2015).

In the third stage of development, seeds go through a pre-programmed period of desiccation, when they are orthodox or are dispersed with high water content when they are recalcitrant. In the Annonaceae family, most of the reports are of species with orthodox characteristics (Table 1). However, some seeds require further studies for their classification, such as those of *A. emarginata*, which present during maturation reduction of water content and the presence of mechanisms of desiccation tolerance (accumulation of sugars and proteins of late embryogenesis-LEA) and allow the maintenance of high germination (70%) when the water content is reduced to 5%, but storage does not exceed 60 days, which may be indicative of intermediate species (CORSATO, 2014).

Mature seeds are dispersed dormant or quiescent. When quiescent, favorable environmental conditions guarantee the germination process, which begins with the acquisition of water and ends with the protrusion of the primary root (BEWLEY et al., 2013).

The acquisition of water by seeds occurs according to the three-phase pattern (BEWLEY et al., 2013), which in annonaceous has the peculiarity of occurring slowly. The first phase (PHASE I) of water acquisition, called imbibition, varies from 5 hours in *A. squamosa* seeds, 36 to 47 h in *Annona* x *atemoya* seeds, 50 h in *A. macrophyllata* and 70 h in *A. purpurea* and *A. emarginata* (reviewed in FERREIRA et al., 2014). This period not only helps detecting the integument impermeability but has been used to determine seed treatments in immersion with plant regulators, or for osmotic conditioning, since it is the period of greatest water acquisition by seeds (GIMENEZ et al., 2014; FERREIRA et al., 2014).

In phase II, water acquisition is slow and dependent on the degradation of reserves. In stage III, a substantial increase of water acquired due to the embryo growth is observed, whose emergence of the primary root ends the germinative process. In annonaceous plants, the protrusion of the primary root takes from 9 to 12 days in *A. macrophyllata* and *A. purpurea* seeds (FERREIRA et al., 2014, GONZÁLEZ-ESQUINCA et al., 2015), and 15-20 days in *A. emarginata* seeds (GIMENEZ et al., 2011).

The seeds of several annonaceous trees present dormancy, which by definition is the inability of viable seeds to germinate in a specific period of time under

optimal environmental conditions (BASKIN and BASKIN, 2014). Reports of dormancy in the family involve mainly morphological and physiological characteristics of the embryo, with some factors related to the integument (Table 1).

Regarding the embryo morphology, the presence of small embryos is characteristic of seeds of this family, being reported with dimensions from 1.2 mm in length in *A. emarginata* (CORSATO et al., 2012) to 3.6 mm in *A. Squamosa* L. (MARTÍNEZ et al., 2013). Size does not necessarily mean they are undifferentiated or dormant. *Annona squamosa*, *A. reticulata* and *A. emarginata* present small differentiated embryos in hypocotyls and cotyledons without dormancy (RIZZINI, 1973; CORSATO et al., 2012); on the other hand, *A. crassiflora*, *A. macrophyllata* and *A. purpurea* present embryos similar to the former, but germination occurs only after 5 to 9 months, which characterizes the physiological component of dormancy (DA SILVA et al., 2007; GONZALEZ-ESQUINCA et al., 2015).

Table 2 shows treatments for overcoming dormancy of anonaceous plants, in most cases with the use of plant regulators, either alone or in mixtures. *Annona macrophyllata*, *A. purpurea* and *Xylopia aromatica* seeds respond to the application of isolated plant regulators such as GA<sub>3</sub>, although they present higher germination rates when mixtures are used to overcome dormancy (200 to 250 mg L<sup>-1</sup> of GA<sub>4+7</sub> + benziladenine), with germination rate of 77%, 30% and 63%, respectively (SOCOLOWSKI, CICERO, 2011; FERREIRA et al., 2016).

It should be noted that phyto regulators are used in some cases as in *A. emarginata* (CORSATO et al., 2012) and *A. x atemoya* (BRAGA et al., 2010) to synchronize and / or increase germination rate.

In relation to the dormancy characteristics related to the integument, it has been demonstrated that Annonaceae seeds are not impermeable, not characterizing this factor as dormancy mechanism (FERREIRA et al., 2014). It is noteworthy that the slow imbibition may be responsible for the uneven germination. In this case, the integument removal can aid the entry of water and oxygen regulators in seeds, in the same way that it can facilitate the elimination of inhibitory substances, favoring the germinative process (DALANHOL et al., 2014). However, they may also result in negative effects as in *A. macrophyllata*, the removal of the micropylar plug from seeds led to increased contamination by pathogenic organisms, perhaps because water acquisition was faster and contamination did not allow embryo germination (GONZÁLEZ-ESQUINCA et al. Al., 2015).

### Grafting in Annonaceous plants

Propagation by grafting aims at the formation of a plant with canopy obtained from commercially productive clones and rootstock tolerant to biotic stress factors (pests

and diseases) and abiotic stress factors (lack or excess of water, soil salinity), which requires union techniques that favor the formation of meristematic tissues in the grafted region and their differentiation into a functional vascular system (LEMOS, 2013). In annonaceous plants, for the union of parts, the main grafting methods are 'budding' and 'cleft' (BARON et al., 2016).

For parts to reestablish the new individual, there are several factors involved that deserve to be studied, such as hormone synthesis (ALONI et al., 2010), phenolic compounds, the activity of antioxidant enzymes and the expression of genes involved in tissue formation (PINA; ERREA, 2008 cited by BARON et al., 2016). For anonaceous plants, researches reveal that compatible combinations present greater expression of the UGP gene soon after grafting, which is involved with the synthesis of tissues in the grafted region (BARON et al., 2016). Thus, the choice of the rootstock should consider not only reactions caused by the canopy / rootstock combination soon after grafting but also photosynthetic responses and nutritional needs.

For *Annona x atemoya* the most commonly used rootstock is *Annona emarginata* (var. terra-fria) a plant tolerant to root rot, trunk drill, and tolerance to dry and flooded soils. As for stress due to lack of water, the species shows recovery of gas exchange and the activity of the enzymes superoxide dismutase and peroxidase, without the detection of lipid peroxidation after 38 days without irrigation (MANTOAN et al., 2016). In addition, there are no changes in the photochemical efficiency between plants maintained under irrigation and rehydrated after stress establishment, indicating photochemical apparatus tolerant to water stress (MANTOAN et al., 2015).

In relation to tissue formation in the grafted region, both araticum (terra-fria variety), araticum mirim (*A. emarginata*, mirim variety), and biribá (*Annona mucosa*) were shown to be compatible with atemoya. After grafting on araticum terra-fria variety, there is an increase in the relative expression of the UGP gene, which indicates a faster tissue formation in the grafted region (BARON et al., 2016), since the gene is involved with cell wall synthesis and was suggested as a candidate in the process of compatibility among woody species (PINA; ERREA, 2008 cited by BARON et al., 2016). This faster formation of tissues in the grafted region reinforces the compatibility and use of the species for atemoya. On the other hand, *A. mucosa* presented about 100% establishment, being the formation of tissues and expression of the UGP gene in the grafted region similar to that observed in araticum terra fria variety. When atemoya was grafted on araticum-mirim, there was a reduction of the relative expression of the UGP gene and a smaller size of nursery seedlings compared to the combination of atemoya grafted on biribá and araticum terra fria variety (BARON et al., 2016). *A. squamosa* and *A. emarginata* are considered alternatives for grafting with

atemoya under climatic conditions at high temperatures, although *A. squamosa* does not show tolerance to root diseases (KAVATI, 2013). *A. cherimola* could also be an alternative to subtropical or tropical altitude conditions; however, its use is discarded because it is susceptible to anthracnose (*Coleotrichum gloeosporioides*), annoneaceous-rust (*Phakopsora neocherimoliae*) and rot of branches (*Botryodiplodia theobromae*) (JUNQUEIRA; JUNQUEIRA, 2014).

In order to graft *A. squamosa*, the use of several rootstocks, such as *A. cherimola*, *A. glabra* (araticum-dobrejo), *A. montana* (falsa-graviola), *A. reticulata* (frutada-condessa), *A. muricata*, *A. senegalensis* (graviola silvestre), *A. x atemoya* (KAVATI, 2013), has been reported. *A. reticulata* provides greater canopy vigor, tolerance to rot and drill (*Heilipus* sp.) in trunk and roots (MANICA, 2003). However, there is differentiated growth in the grafted region, with “elephant leg” formation. Grafting pine cone in *A. mucosa* was incompatible, with high mortality at 45 days after grafting and establishment between 4 and 19.2% (SANTOS et al., 2005). Species such as *Annona neosalicifolia* should be evaluated for locations with high temperatures (KAVATI, 2013).

For *A. muricata*, the most recommended rootstocks are graviola, *A. reticulata*, *A. montana*, *A. mucosa* and *A. glabra* (KAVATI, 2013). When graviola was grafted onto itself, *A. montana*, *A. glabra* and *A. mucosa*, the percentage of successful grafts was equal to or greater than 90%, and grafting on graviola or *A. montana* resulted in a greater length and diameter of branches and number of leaves and the combination with *A. glabra* showed the worse development, giving indications of the dwarfing characteristic that is demonstrated in the field (CARVALHO et al., 2000).

The grafting of *A. cherimola* has been carried out mainly in cherimoia itself (PADILLA; ENCINA, 2011), because as cultivation must be under conditions of subtropical climate without frost or tropical attitude, the number of species to be used as rootstock is quite restricted. Alternative has been *A. emarginata* (terra-fria variety) for presenting resistance at low temperatures.

### Cutting in Annonaceous plants

The maintenance of the genetic characteristics among generations of allogamous plants is only possible through the use of asexual propagation techniques. The cutting technique is one of the most common, simple and fast methods of cloning woody fruit trees, and it is based on the ability of an organ of a plant (stem, leaf or root) to regenerate another complete plant; however, the rooting process is very complex, whose genes and molecular mechanisms that respond for the formation of adventitious roots have not yet been fully identified (HARTMANN et al., 2011). The cutting technique in annonaceous plants has been studied for several decades, being previously

described as a very limited success practice, because it is a group of species with difficult rooting (SANTOS et al., 2011).

In *A. x atemoya*, the rooting ability of cuttings appears to be cultivar-dependent. Studies with African Pride cultivar showed a success rate of 15% higher than Pink's Mamooth of 5% (SANIEWSKI, 1988). Ferreira and Ferrari (2008) obtained with Gefner cultivar more than 80% of the rooting in the surviving cuttings.

The formation of adventitious roots can occur directly from the multiplication of meristematic cells around the vascular cambium. In the indirect formation, root primordia begin with callus tissues that form first and evolve to a connection with the vascular system, which is a typical characteristic of species of difficult rooting (HARTMANN et al., 2011).

Longitudinal histological cuts performed at the bases of *Annona squamosa* cuttings revealed that the radicular primordia always arise from parenchymal tissues initiated from the region of the vascular cambium. In this species, from 15 days, root primordia could already be observed, and the roots could be externally visible from 25 days (LEMOS, BLAK, 1996). For atemoya, roots appear between 8 and 12 weeks (FERREIRA; FERRARI, 2010).

The formation of calli at the base of cuttings is a fact independent of root induction, many rooted cuttings are poor or without calli. In some annonaceous such as *Annona glabra* and *A. montana*, the early appearance of roots prevented the development of calli on cuttings, which showed roots in quantity and quality to ensure good establishment of seedlings (SCALOPPI JUNIOR et al., 2007).

Soft temperatures favor the formation of calli, and higher temperatures rooting. In experiments with *A. glabra* and *A. montana*, the lowest rooting in the autumn (20% and 8% respectively) and winter months (4% and 14%) was related to the higher production of calli in cuttings, while in the summer months, rooting was 94% and 48%, accompanied by a small presence of calli (SCALOPPI JÚNIOR; MARTINS, 2003).

The effect of age is fundamental in the promotion of rooting on cuttings of young *Annona cherimola* (25%) and *Annona mucosa* plants (40%) when compared to cuttings of adult plants without rooting (SCALOPPI JR.; MARTINS, 2014). A similar result was described by Geneve et al. (2007) with pawpaw (*Asimina triloba*), only cuttings of plants less than two months old treated with various Indole Butiric Acid (IBA) concentrations by rapid immersion were able to rooting.

The age of plants is not an impediment to the rooting of cuttings when adult plants are suitably conditioned for the removal of propagules. In *A. cherimola*, Encina and González-Padilla (2011) observed that micro-cuttings collected from adult ‘Fino de Jete’, ‘Bonita’ and ‘Pazicas’ plant cultivars had very poor rooting but

could be rejuvenated through sequential grafting. After three consecutive grafts, rooting percentages increased significantly up to 70%. For *A. squamosa* (SALVADOR et al., 2014), *A. muricata* (SANTOS et al., 2013) and *A. x atemoya*, (FERREIRA; FERRARI, 2010) rooting of cuttings of adult plants was possible when propagules were removed from young vigorous shoots of pruned plants (sugar apple and atemoya) or plants of a clonal garden in a permanent vegetative state through frequent pruning (*A. muricata*).

A strategy widely used in propagation by cutting is the use of auxins to stimulate rooting, whose effectiveness is IBA>NAA>IAA (Indole Butiric Acid > NaphthalenAcetic Acid > Indole Acetic Acid) individually applied (KESARI et al., 2009). The method of IBA application in the form of powder showed to be significantly superior to the liquid form in *A. squamosa*, presenting 86% of rooting of cuttings against 66% for the same IBA concentration used (SALVADOR et al., 2014). Better responses in the form of powder were also obtained for *A. muricata* (SANTOS et al., 2011). The greater efficiency of the solid form in the application of auxins seems to be related to a longer and moderate exposure of the hormone to target tissues, once auxin crystals are insoluble in water and, in contact with the wound tissues and cutting exudates are slowly dissolved.

The presence of leaves on the cuttings and, consequently, the higher rooting index of *Annona emarginata* were strongly influenced by the spray system combined with the spray frequency and droplet size in the maintenance of relative air humidity (SCALOPPI JÚNIOR, 2007). Ultrasonic nebulizers were the most recommended to maintain high relative humidity and reduce transpiration and the fall of *A. muricata* leaves (MARINHO et al., 2007; SANTOS et al., 2011).

Herbaceous or semi-woody cuttings are preferred for annonaceous cuttings; however, only cuttings obtained from vigorous shoots are properly rooted. Adult or decaying trees do not always produce such sprouts, but they can be induced by canopy pruning. Sub-apical cuttings obtained from vigorous shoots of *A. squamosa* pruned at 12 years of age, maintained with one or two pairs of leaves cut in half, had rooting rates higher than 80% (SALVADOR et al., 2014). Vigorous branches of the green pruning of atemoya performed with the function of promoting greater aeration and decrease vigor are the best for the cutting practice (SCALOPPI JÚNIOR; MARTINS, 2014).

### ***In vitro* tissue culture**

*In vitro* tissue culture (micropropagation) is among propagation methods successfully applied to species such as *A. cherimola*. Advances in this area are presented in a recent literature review by Encina et al. (2014), who reported micropropagation with juvenile *A. muricata* material and protocol for micropropagation of adult *A. cherimola* genotypes. The authors also presented several *in vitro* methodologies, such as adventitious organogenesis and regeneration of cell cultures; manipulation of the 'ploidy' of *A. cherimola* to obtain haploid, tetraploid, triploid (seedless) plants; genetic transformation with introduction of genes aiming to control the post-harvest processes and to provide resistance to pathogens and insects; and micropropagation and regeneration of other wild species of the genus *Annona*, such as *A. senegalensis*, *A. scleroderma*, *A. montana*, among others.

**Table 1.** Seed biological characteristics from Annonaceae species.

Species	Storage behavior	Germination strategy	References
<i>Annona acuminata</i> Saff.	Orthodox	No dormancy	Daws et al, 2005
<i>Annona cacans</i> Warm.		Dormancy: Physiol	Dalanhol et al, 2013
<i>Annona cherimola</i> Mill.	Orthodox	Dormancy	RBG Kew, 2016
<i>Annona coriacea</i> Mart.		Dormancy: Morphophys	Dresch et al, 2014
<i>Annona crassiflora</i> Mart.	Orthodox	Dormancy: Physiol	Da Silva, 2007
<i>Annona emarginata</i> (Schltdl.) H.Rainer	Intermediate	No dormancy	Corsato et al, 2012
<i>Annona glabra</i> L.	Orthodox	No dormancy	RBG Kew, 2016
<i>Annona hayesii</i> Saff.	Orthodox		RBG Kew, 2016
<i>Annona hypoglauca</i> Mart.		Dormancy	Parolin et al, 2003
<i>Annona macrophyllata</i> Donn Sm.	Orthodox	Dormancy: Physiol	González-Esquincia et al, 2015
<i>Annona montana</i> Macfad.		No Dormancy	Oliveira et al, 2005
<i>Annona mucosa</i> (Jacq.) Baill. H. Rainer	Orthodox?	No Dormancy	Ferreira et al 2009
<i>Annona muricata</i> L.	Orthodox		RBG Kew, 2016
<i>Annona purpurea</i> Moc. & Sessé ex Dunal	Orthodox	Dormancy: Physiol	González-Esquincia, 2016, Ferreira et al, 2016.
<i>Annona reticulata</i> L.	Orthodox	No Dormancy	RBG Kew, 2016
<i>Annona spraguei</i> Saff.	Orthodox	Dormancy: Morphophys	RBG Kew, 2016
<i>Annona squamosa</i> L.	Orthodox	?	RBG Kew, 2016
<i>Annona sylvatica</i> A.St.-Hil		Dormancy: Morphophys	Mayer et al, 2008
<i>Annona x atemoya</i> Mabb.		Dormancy: Physiol	Gimenez et al, 2012
<i>Asimina obovata</i> (Willd.) Nash	Orthodox	Dormancy	Menges et al, 2012
<i>Asimina parviflora</i> Dunal	Uncertain		RBG Kew, 2016
<i>Asimina triloba</i> Dunal	Recalcitrant	Dormancy: Physiol	Finneseth et al 1998
<i>Cymbopetalum baillonii</i> R.E.Fr.	Uncertain	No Dormancy	RBG Kew, 2016
<i>Dennettia tripetala</i> Baker f.	Orthodox	No Dormancy	Nwachukwu et al 2010
<i>Friesodielsia obovata</i> (Benth.) Verdc.	Orthodox		RBG Kew, 2016
<i>Goniothalamus amuyon</i> (Blanco) Merr.		No Dormancy	Chen et al, 2015
<i>Guatteria australis</i> A.St.-Hil		Dormancy: Physiol	Goncalves et al, 2006
<i>Monodora myristica</i> (Gaertn) Dunal	Orthodox		RBG Kew, 2016
<i>Polyalthia longifolia</i> (Sonn.) Thwaites	Recalcitrant?		RBG Kew, 2016
<i>Stelechocarpus burahol</i> (Blume) Hook.f. & Thomson	Recalcitrant?		RBG Kew, 2016
<i>Unonopsis guatterioides</i> (ADC) R.E.Fr		Dormancy: Morpho	Battilani et al 2007
<i>Uvaria acuminata</i> Oliv.	Orthodox		RBG Kew, 2016
<i>Uvaria brevistipitata</i> De Wild.	Orthodox		RBG Kew, 2016
<i>Uvaria chamae</i> P.Beauv.	Orthodox		RBG Kew, 2016
<i>Xylopia aethiopica</i> (Dunal) A.Rich.	Orthodox		RBG Kew, 2016
<i>Xylopia aromaticata</i> (Lam.) Mart.	Orthodox	Dormancy: Physiol	Socolowski & Cicero, 2011
<i>Xylopia frutescens</i> Aubl.		Dormancy: Morphophys	Sautu et al, 2007
<i>Xylopia sericea</i> A.St.-Hil.		Dormancy: Physiol	Santos, 1994 in Socolowski & Cicero, 2011

\*Seed storage behaviour refers to the capacity of seeds to survive desiccation. Germination strategy: Physiological (Physiol), Morphological (Morpho); Morphophysiological (Morpho-Physiol)

**Table 2.** Seed germination of Annonaceae species

Species	Treatment	%GT	%GC	References
<i>Annona cherimola</i>	GA <sub>3</sub> , 8.67 uM, 24 h	80	--	Padilla & Encina, 2003
<i>Annona coriacea</i>	GA <sub>3</sub> , 350 mg.L <sup>-1</sup> , 144 h	69	--	Dresch et al, 2014
	Storage burial, 10 months	60	0	Da Silva et al, 2007
<i>Annona crassiflora</i>	GA <sub>4+7</sub> , 500 µM	43	0	Da Silva et al, 2007
	GA <sub>3</sub> , 4000 mg.L <sup>-1</sup> , 73 h, burial in sand	51	--	Cavalcante et al 2007
<i>Annona emarginata</i>	Fresh Seed, GA <sub>4+7</sub> + 6-BA, 250 mg.L <sup>-1</sup> , 60 h	70.5	54.5	Corsato et al, 2012
	Seed humidity at 23% H, 20/30°C	27.0	8.5	Costa et al, 2011
<i>Annona glabra</i>	Storage 6 months, mechanical scarification	86	--	Zamora-Cornelio et al, 2010
	Floatation inside fruit, 3 months	88	55	Setter et al, 2008
<i>Annona hypoglauca</i>	Fresh Seed, Burial	4	0	Parolin et al, 2003
	Seed Storage, 8 months	71	0	González-Esquinca et al, 2015
<i>Annona macrophyllata</i>	GA <sub>3</sub> , 200 mg.L <sup>-1</sup> , 96 h	51	0	
	GA <sub>4+7</sub> + 6-BA, 200 mg.L <sup>-1</sup> , 96 h	77	0	Ferreira et al 2016
<i>Annona montana</i>	Fresh Seed, 30°C vs 25°C	55	25	Oliveira et al, 2005
<i>Annona mucosa</i>	Scarification	76	58	Ferreira et al, 2009
	Sand burial	75	19	Dos Santos et al, 2005
<i>Annona muricata</i>	Storage 1 month a 6°C, acid scarification	60	--	Oumar et al 2012
	Storage 1 month a 6°C, Shelled seeds	63	--	
	Acid scarification, 1 min	77	71	Meza and Bautista, 2004
<i>Annona purpurea</i>	GA <sub>3</sub> , 500 mg.L <sup>-1</sup> , 96 h	29	4	
	GA <sub>4+7</sub> + 6-BA, 200 mg.L <sup>-1</sup> , 96 h	30	1	Ferreira et al ,2016
	Storage 6 months, GA <sub>3</sub> , 1000 mg.L <sup>-1</sup> , 120 h	68	8	Gómez-Castañeda et al, 2003
<i>Annona senegalensis</i>	Storage 1 month a 6°C, shelled seeds	60	6	
	Storage 1 month a 6°C, acid scarification	44-58	--	Oumar et al, 2012
<i>Annona spraguei</i>	Fresh seed, burial in sand, 25–31°C, 30% sunlight	15	--	Sautu et al, 2006

Species	Treatment	%GT	%GC	References
<i>Annona squamosa</i>	GA <sub>3</sub> , 50 mg.L <sup>-1</sup> , 24 h	75	3.8	Stenzel et al, 2003
	CK + GA <sub>3</sub> + IBA 750 mg.L <sup>-1</sup> , 12 h	98	3	Sousa et al, 2008
	Scarification + GA <sub>3</sub>	50	4	Vasconcelos et al, 2015
	room Temperature storage, GA <sub>3</sub> , 400 mg.L <sup>-1</sup>	84	--	Vidal-Lezama et al, 2011
	room T storage, 60 and 120 days	80	--	Martínez et al, 2015
	room T storage, 7 days	33	--	Wagner Junior et al, 2006
	Storage 1 month 6°C, Shelled seeds	73	25	Oumar et al, 2012
	Storage 1 month 6°C, acid scarification	44-58	--	Oumar et al ,2012
	Scarification + immersion in water for 6 h	80	42.5	Chagas et al, 2013
	Scarification, + GA <sub>3</sub> 1000 mg L <sup>-1</sup> 24 h	72.5	42.5	
	Zeatin 1 mg L <sup>-1</sup> , 48 h	70	24	Moreno et al 2013
	Fresh Seed, Germination a 30°C vs 18.7°C	58	10	Martínez et al, 2012
<i>Annona x atemoya</i>	Fresh Seed, GA <sub>3</sub> , 400 mg.L <sup>-1</sup> , 72 h	92	9	Martínez et al, 2016
	GA <sub>3</sub> , 100 mg.L <sup>-1</sup> , 12 h	60	28	
	KNO <sub>3</sub> 2%,12 h	47	28	Parmar et al, 2016
	Thiourea 1000 mg.L <sup>-1</sup> , 12 h	51	28	
	GA <sub>4+7</sub> + 6-BA, 300 mg.L <sup>-1</sup> , 32 h	44	5	Gímenez et al, 2012
	GA <sub>3</sub> , 778 mg.L <sup>-1</sup> , 36 h	85.5	58	Oliveira et al, 2010
CV Gefner	GA <sub>3</sub> , 520 mg.L <sup>-1</sup> , 144 h	89	55	
	GA <sub>4+7</sub> + 6-BA, 520 mg.L <sup>-1</sup> , 36 h	95.5	58.5	Braga et al, 2010
	CK+GA+IBA, 2.8 mg.Kg <sup>-1</sup>	51	50	
CVPR-1	GA <sub>3</sub> , 50 mg.L <sup>-1</sup> , 24 h	36	1	Stenzel et al, 2003
CVPR-3	GA <sub>3</sub> , 50 mg.L <sup>-1</sup> , 24 h	61	1	Stenzel et al, 2003
<i>Asimina obovata</i>	Fresh Seed, mechanical scarification	12	7	Menges et al, 2012
<i>Asimina parviflora</i>	Germination in agar; 25°C, photoperiod 8/16 h	76		RBG Kew, 2016
<i>Asimina triloba</i>	Chilling stratification	84-90	12	Finneseth et al, 1998
<i>Cymbopetalum baillonii</i>	Forest shade environment	92	44	Coates-Estrada, 1988
<i>Dennettia tripetala</i>	Fresh Seed	79.8	--	Nwachukwu et al 2010
<i>Friesodielsia obovata</i>	Seed scarified (chipped with scalpel), agar; 25°C, photopheriod 8/16	100	--	RBG Kew, 2016
<i>Goniothalamus amuyon</i>	Fresh Seeds, 30°C, light, 4 weeks <b>vs 15/5°C</b>	70.0	0	
	Fresh Seeds, 30°C, light, 8 weeks <b>vs 15/5°C</b>	97.0	0	Chen et al, 2015
<i>Guatteria australis</i>	Germination in obscurity	28.1	12.5	Goncalves et al, 2006
<i>Unonopsis guatterioides</i>	Germination in green house (Photoblastic)	70	3	Battilani et al 2007
<i>Uvaria acuminata</i>	Seed scarified (chipped with scalpel), agar; photopheriod 8/16; <b>20°C vs 25°C</b>	100	88	RBG Kew, 2016

Species	Treatment	%GT	%GC	References
<i>Xylopia aromatica</i>	GA <sub>4+7</sub> + 6BA 500 mg.L <sup>-1</sup> , 48 h	47.5	0	
	GA <sub>4+7</sub> + 6BA 250 mg.L <sup>-1</sup> , 48 h; seeds without aril & sacotesta	63.0	0	
	GA <sub>4+7</sub> + 6BA 500 mg.L <sup>-1</sup> , 48 h in sand	70.0	0	
	GA <sub>4+7</sub> + 6BA 250 mg.L <sup>-1</sup> , 48 h, in "Cerrado Soil; seeds without aril & sacotesta	57	0	
	GA <sub>4+7</sub> , 250 mg L <sup>-1</sup> , 48 h, burial in sand; seeds without aril & sacotesta	59	0	Socolowski and Cicero, 2011
	GA <sub>4+7</sub> , 500 mg L <sup>-1</sup> , 48 h, in "Cerrado" Soil; seeds without aril & sacotesta	67	0	
	GA <sub>4+7</sub> + 6BA 500 mg.L <sup>-1</sup> , 48 h. Storage 8 months	80	53	
<i>Xylopia frutescens</i>	GA <sub>4+7</sub> + 6BA 500 mg.L <sup>-1</sup> , 48 h. Storage 7 months	67	46	
	GA <sub>4+7</sub> + 6BA 500 mg.L <sup>-1</sup> , 48 h. Seed fresh	70	2	
	Fresh seed, burial in sand, 25–31°C, 30% sunlight	1.3	--	Sautu et al, 2006
	Fresh seed, burial in sand, 25–31°C, 30% sunlight	9	--	Sautu et al, 2006

\*% GT: Percentage of Germination or Emergency with treatment; % GC: Percentage of Germination or Emergency without treatment (Control).  
-- Data not shown; GA<sub>3</sub> = gibberellic acid; GA4+7 = Giberelins 4 and 7; 6-BA = 6 benzyladenine; CK = Kinetin; AIB = Indol-butrylic acid

## Conclusion

There are a number of studies that have characterized the processes of propagation of 38 annonaceous, including the five most cultivated species, which means that in 98% of species of the family, the physiological aspects reported in this article are unknown. The major challenge remains to characterize the mechanisms involved in desiccation tolerance seeds and the physiological factors of dormancy seed, since mature seeds are dispersed with differentiated dormant or not embryos. Regarding grafting, although advances have been made in relation to compatible combinations, further studies should be directed to understand the physiological mechanisms of the process, including the influence that the rootstock exerts on the gaseous exchanges, mineral nutrition and canopy productivity. Cutting is the least used propagation method in Annonaceae due to the rooting difficulty, which can be reversed when advances already obtained experimentally are incorporated into the protocols for seedling production.

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