

## Technological innovations in the propagation of Açai palm and Bacuri

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**Abstract-** Açai palm (*Euterpe oleracea* Mart.) and bacuri tree (*Platonia insignis* Mart.) are two important fruit species native to the Brazilian Amazon. The first species is widely cultivated in the Brazilian Amazon and with some orchards established in other regions of Brazil. Nevertheless, most of its production still comes from the dense and diversified native açai orchards found in the floodplains of the Amazon River estuary and that in the last two decades have been managed for the production of fruits. The production of the second species is still dependent on extractivism of abundant natural populations in areas of secondary vegetation. Both species reproduce naturally sexually and asexually. In the case of açai tree, asexual reproduction occurs by means of the emission of tillers in the base of stems. For bacuri tree, reproduction is verified by means of abundant sprouts that arise from roots that develop horizontally, near the ground surface. The propagation of açai palm tree, particularly sexually is a consolidated process, practically without innovations in the last years. Açai seeds show rapid and relatively uniform germination. A seedling obtained from seeds is suitable for final planting four to six months after emergence of seedlings. Regarding propagation by asexual route, it has been demonstrated that tillers can be used for the production of seedlings. For this, they should be separated from the mother plant when they have two completely expanded leaves and one at the beginning of formation. The big challenge is to increase the multiplication rate of tillers. For bacuri tree, considerable advances have been obtained both for sexual and asexual propagation. Propagation by primary root cuttings or direct seeding at the definitive site constitutes innovations that overcome the problem of the slow and uneven germination of the bacuri seed. The developed grafting techniques allow both the production of grafted seedlings and grafting in the field, for plants from direct seeding.

**Index terms:** *Euterpe oleracea*, germination, grafting, *Platonia insignis*.

## Inovações tecnológicas na propagação do Açaizeiro e do Bacurizeiro

**Resumo-** O açaizeiro (*Euterpe oleracea* Mart.) e o bacurizeiro (*Platonia insignis* Mart.) são duas importantes espécies frutíferas nativas da Amazônia Brasileira. A primeira espécie já é bastante cultivada na Amazônia Brasileira e com alguns pomares estabelecidos em outras regiões do Brasil. Não obstante, a maior parte da produção ainda é oriunda dos densos e diversificados açaiçais nativos encontrados nas várzeas do estuário do Rio Amazonas e que, nas últimas duas décadas, vêm sendo manejados para a produção de frutos. A produção da segunda espécie também ainda é dependente do extrativismo de populações naturais abundantes em áreas de vegetação secundária. Ambas as espécies reproduzem-se naturalmente por via sexuada e assexuada. No caso do açaizeiro, a reprodução assexuada se dá-se por meio da emissão de perfilhos na base dos estipes. Já no bacurizeiro, verifica-se por meio de abundantes brotações que surgem de raízes que se desenvolvem horizontalmente, próximas à superfície do solo. A propagação do açaizeiro, particularmente por via sexuada, é um processo já consolidado, praticamente sem inovações nos últimos anos. As sementes de açai apresentam germinação rápida e relativamente uniforme. Uma muda obtida a partir de sementes está apta para o plantio no local definitivo entre quatro e seis meses após a emergência das plântulas. No que se refere à propagação por via assexuada, foi demonstrado que os perfilhos podem ser utilizados para a produção de mudas. Para tanto, devem ser separados da planta-mãe quando apresentarem duas folhas completamente expandidas e uma em início de formação. O grande desafio consiste em aumentar a taxa de multiplicação dos perfilhos. Com relação ao bacurizeiro, consideráveis avanços foram obtidos tanto no que se refere à propagação sexuada como a assexuada. A propagação por estacas de raiz primária ou a semeadura direta no local definitivo constituem-se em inovações que contornam o problema da germinação lenta e desuniforme da semente de bacuri. As técnicas de enxertia desenvolvidas possibilitam tanto a produção de mudas enxertadas como a enxertia no campo, para plantas oriundas de semeadura direta.

**Termos para indexação:** enxertia, *Euterpe oleracea*, germinação, *Platonia insignis*.

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## Introduçõ

In the Brazilian Amazon, approximately 220 fruit species are found, of which few have until then assumed a prominent position in regional fruit growing market (CARVALHO, 2012). Many of these species are not cultivated because they have a long young phase, requiring, in many cases, periods longer than ten years to enter the fruiting phase. In addition, some of them present seeds with complex dormancy mechanisms, which implies slow germination and with marked unevenness, making seedling production difficult.

In the specific case of açai palm tree, the species is already widely cultivated, even outside the Brazilian Amazon, despite the fact that more than 90% of the production still comes from the dense and abundant native açai orchards found in the floodplains of the Amazon River estuary and that in the last two decades have been managed for fruit production. Estimates indicate that the areas managed and planted with açai trees only in the State of Pará, occupy 154 thousand hectares (OLIVEIRA, TAVARES, 2016). At present, it is the fruit most produced and consumed in the State of Pará, with production volume around 600 thousand tons of fruit per year (QUEIROZ, 2016).

The production systems of açai tree seedlings developed more than 10 years ago have been efficient and effective, since it is a species of easy propagation, without major phytosanitary problems in the nursery stage and with seeds that germinate quickly, and with seeds that germinate rapidly, being a seedling suitable for planting between six and eight months after sowing (OLIVEIRA et al., 2000; NOGUEIRA et al., 2005). Natural regeneration is also efficient because it presents sexual and asexual reproduction strategies, the latter being by tillers that emerge at the base of the mother plant.

The production of bacuri tree is also still almost totally dependent on extractivism. The species is still little cultivated, because it presents a long young phase, only producing the first fruits in the earliest genotypes between eight and ten years after planting the seedling, when propagated by seeds. In addition, seeds present a dormancy mechanism, which leads to slow and uneven germination (Carvalho et al., 1998).

Paradoxically, in nature the bacuri tree aggressively recolonizes newly deforested areas and even those submitted to successive cutting-burning-fallow-cultivation cycles. This is due to the fact that the species reproduces both sexually and asexually, in the latter case by abundant and vigorous shoots that originate from roots, even after the felling of the mother plant. It is not uncommon to find more than 15,000 bacuri trees at the beginning of regeneration per hectare in areas where secondary vegetation was felled (HOMMA et al., 2007). In this situation, almost all plants come from root shoots,

because the species does not form a seed bank.

At the beginning of the 1940s, Pesce (2009) called attention to the fact that bacuri tree was not cultivated due to its slow growth, although, at that time, fruits had great popular acceptance, being consumed in the fresh form and in the form of juice. They also had acceptance by small sweet factories, widely disseminated in the state of Pará at this time.

The aim of this work was to collect and compile the information available in literature on the propagation of açai palm and bacuri trees, with emphasis on technological innovations. At the same time, data generated in the last years and not yet published will be presented.

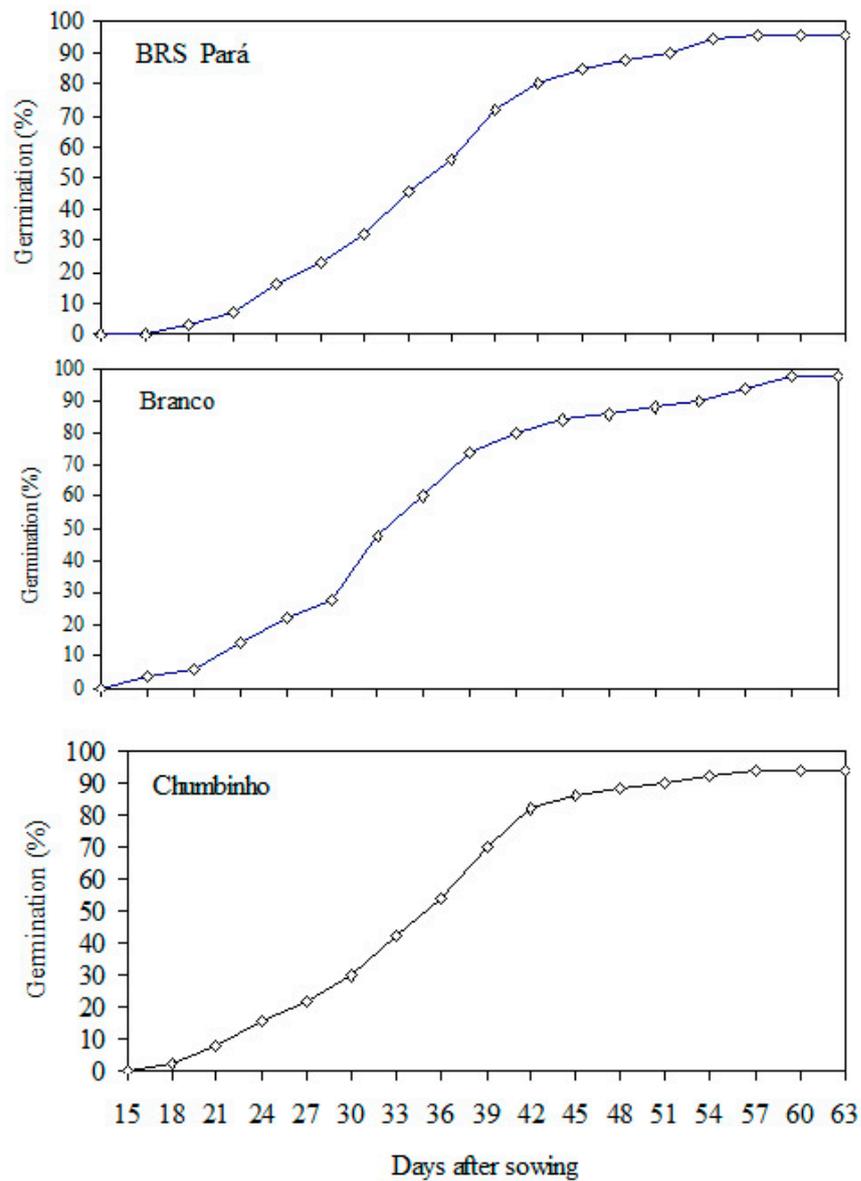
### Propagation of the açai tree

Açai can be propagated sexually and asexually, the latter by tillers that appear at the base of the mother plant. The presence of sexual and asexual reproduction strategies allows the sustainable management of dense and abundant natural populations occurring in the floodplains of the Amazon River estuary, both for fruit production and for palm heart extraction (NOGUEIRA et al., 2005).

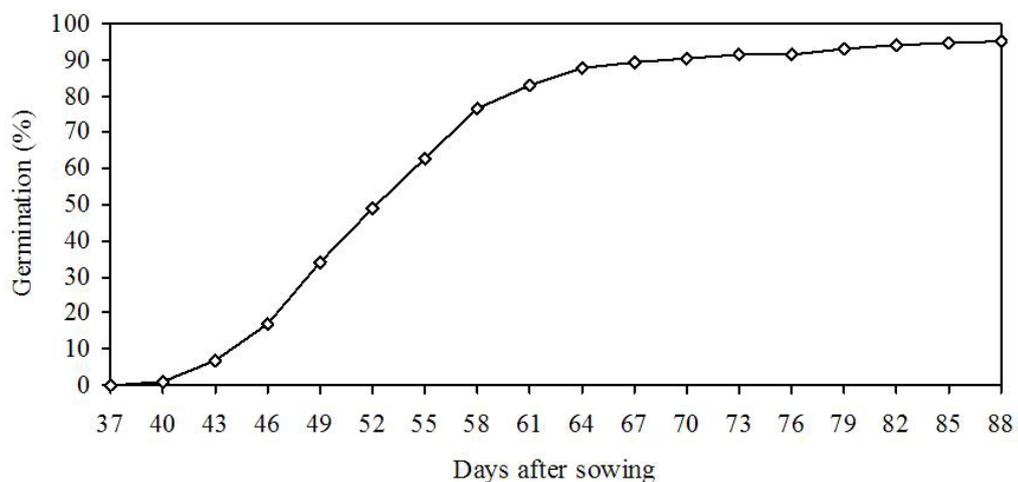
### Sexual propagation

The propagation unit of açai tree is represented by the massive lump (diaspore), which consists of the fibrous part of the mesocarp, the endocarp and the seed. The lump follows the subglobose shape of the fruit and represent between 69.3% and 82.5% of the fruit mass (OLIVEIRA et al., 2000). The seed presents thin integument, tiny embryonic axis and abundant tissue of endospermic reserve rich in lipids. In the BRS Pará cultivar, one kilogram contains, on average, 550 lumps (OLIVEIRA; FARIAS NETO, 2004). In other cultivars popularly known in the Brazilian Amazon such as "chumbinho", which are characterized by the much smaller size of fruits, between 1080 and 1220 lumps per kilogram are found.

Açai seeds (*Euterpe oleracea* Mart.) do not present dormancy mechanisms, and when sown soon after pulping, when the water content of lumps is around 40%, they germinate readily, regardless of genotype, although with a certain lack of uniformity (Figure 1). The uneven germination is probably associated with the fact that seeds, even when coming from a single cluster, do not show uniform maturation. On the other hand Amazon açai (*Euterpe precatoria* Mart.), a single stem palm whose fruits have the same use of the *Euterpe oleracea* Mart species, which also presents edible palm heart, presents seeds with much slower germination, and in this case, it is possible to infer the existence of some dormancy mechanism controlling germination (Figure 2).



**Figure 1** - Germination of açai (*Euterpe oleracea* Mart.) seeds of BRS Pará, açai Branco and Chumbinho cultivars. Source: CARVALHO, J.E.U. de; NASCIMENTO, W.M.O. do. (Unpublished data).



**Figure 2** - Germination of Amazon açai (*Euterpe precatoria* Mart.) seeds as a function of time. Source: CARVALHO, J.E.U. de; NASCIMENTO, W.M.O. do. (Unpublished data).

### Conservation of seed viability

Açaí seeds, as well as of the other species of *Euterpe* generic taxon, present recalcitrant behavior in storage, that is, they do not support desiccation. The germinative power decreases when the water content is reduced to below 30%, but the total impairment occurs when it reaches values close to 15.0% (NASCIMENTO, SILVA, 2005). In addition to desiccation intolerance, seeds are sensitive to low temperatures, with loss of viability when stored at or below 15°C (NASCIMENTO, 2006). For the maintenance of the viability of seeds, it is recommended to pack them in polyethylene packaging and keep them in an environment with temperature of 20°C. This procedure allows storage for up to six months without significant changes in seed germination and vigor (NASCIMENTO, 2006).

At Embrapa Amazônia Oriental, stratification of lumps in substrate moistened in water for the commercialization of seeds has been adopted. The substrate may be vermiculite or even sawdust powder, previously sterilized in boiling water for two hours. For stratification, lumps are mixed with the substrate in the volumetric ratio of one part of substrate to one of seed and packed in plastic bags or polystyrene boxes. In this situation, seeds have favorable conditions for germination, so they should not be kept stratified for more than 15 days, the ideal period being 10 days. From the fifteenth day, the entanglement of the roots of seeds that initiated germination occurs, making it difficult to remove and even causing root breaking.

### Sowing methods

Açaí tree seeds can be sown in sowing containers or directly in containers in which seedlings will be produced. When sowing is used in plastic bags, it is recommended that at least 20% of seeds are placed in sowing containers. This procedure allows only one seed to be sown in each container. Since more than 85% of seedling emergence is commonly obtained, seedlings from sowing containers are placed in containers where seeds have not germinated. For no pronounced damage to occur to the root system under formation, the sowing substrate should be quite friable and the transplantation for the plastic bags should be carried out when plants are in the stick stage (Figure 3). Mixtures of sand with sawdust powder, or sand and coconut fiber, in the volumetric ratio of 1: 1, are substrates suitable for this purpose.

For seedlings marketed four to eight months after emergence, it is recommended to use polyethylene bags with minimum size of 15 cm in width and 25 cm in height, with thickness of 100  $\mu$ . A good substrate for seedling production consists of mixing soil with sawdust powder and manure at volumetric ratio of 3: 1: 1. For seedlings aged eight to twelve months, polyethylene bags 17 cm in width and 27 cm in height should be used, with thickness

of 100  $\mu$ . Larger containers, 18 cm in width and 35 cm in height, and flexible plastic pots with capacity of 7 liters are recommended for seedlings aged over 12 months. These last two containers keep seedlings in good condition for 18 and 24 months, respectively.

Some nurserymen from the state of Pará are producing açaí tree seedlings in tubes. In this case, they use as substrate a mixture of crushed coconut fiber (840 liters), clay (80 liters) and carbonized rice husk (80 liters), enriched with organic and chemical fertilizers. Each cubic meter of the mixture of crushed coconut fiber, soil and carbonized rice husk is added of 4 kg of castor oil cake, 4 kg of rice flour, 2 kg of dolomitic limestone, 1 kg of Yorin Master and 2 kg of NPK fertilizer, 14-14-14 formulation, slow release. Although nurserymen use tubes with capacity of 110 cm<sup>3</sup> or 175 cm<sup>3</sup> of substrate, the ideal condition is that seedlings are produced in larger tubes, with capacity of 260 cm<sup>3</sup>, which allows the commercialization of seedlings up to eight months old. In the field, especially in irrigated açaí orchards, seedlings produced in tubes show growth similar to that of seedlings produced in polyethylene bags.

### Asexual propagation of açaí trees

The asexual propagation of açaí tree is possible because this palm tree is capable of tillering, being not rare to find clumps with up to 45 stems in different growth stages. However, it should be emphasized that, within the species, there are individuals that do not show or exhibit low tillering capacity, emitting no more than two or three stems during their life cycle. At the Embrapa Amazonia Oriental Açaí Germplasm Bank, it was verified that 19% of plants did not exhibit tillering capacity, exhibiting the characteristics of single stem plant (OLIVEIRA et al., 2015). Tillering begins four to six months after planting of the seedling at the definitive site and when the stem enters the fruit production phase, it loses the ability to emit tillers. The sexual propagation of açaí tree is important because it is a species with high alogamy rate (OLIVEIRA et al., 2015).

### Propagation by tillers

In asexual propagation, the first step involves the separation of the tiller from the mother plant. This operation must be carried out in the rainy season, with the aid of a sharp iron wedge. The tiller should be removed with a small amount of roots. Very large tillers are difficult to separate from the mother plant and have low survival capacity. Ideally, they should have two completely expanded leaves and one at the beginning of formation (Figure 4).

After removal, they should be planted in plastic bags with minimum dimensions of 18 cm in width by 35 cm in height or preferably in flexible plastic containers with capacity of seven liters of substrate. In order to have good survival rater, it is imperative that after planting,

seedlings are kept in nursery with intermittent misting system and 50% shading. The adoption of these procedures allows the conversion rate of tillers into seedlings suitable for planting of at least 65%. Seedlings propagated by tillers are fully formed and capable of being planted in the field four months after being placed in the propagator (NASCIMENTO et al., 2011). The main problem for the commercial use of seedlings obtained by this method is the reduced number of tillers that can be removed from a mother plant.

### ***In vitro* propagation**

Works developed so far on the *in vitro* propagation of açai tree have been more successful with the cloning of zygotic embryos, which has little interest in the large-scale production of seedlings. These studies serve only as a model for the development of protocols for somatic embryogenesis (SCHERWINSKI-PEREIRA, 2012; OLIVEIRA et al., 2015, FREITAS et al., 2016). For somatic embryogenesis, promising results were obtained from embryogenic calluses originating from inflorescences and immature leaves. In the first case, 100% of the explants differed in embryos, which progressed slowly to the torpedo stage. In the second case, the differentiation was only 50%, and embryos did not reach higher development stages (FREITAS, 2014).



**Figure 3** - Açai seedling (*Euterpe oleracea* Mart.) at the stick stage.



**Figure 4** - Açai tree clump (*Euterpe oleracea* Mart.) with tillers at the ideal condition to be removed for seedling production.

### Bacuri tree propagation

Bacuri tree can be propagated sexually and asexually. When the objective is fruit production, seed propagation has as limitation the fact that the species presents a long young phase and is essentially allogenic due to the existence of genetic self-incompatibility mechanism (MAUÉS, VENTURIERI, 1996; CARVALHO, MÜLLER, 2007). For plantations with logging purpose, sexual propagation is indicated, since plants from seed show faster growth, form straight stem, with natural pruning and reach more than 25 m in height over 20 years of age. In turn, grafted plants are small in size, with height of less than ten meters, tortuous stem and without natural pruning.

### Seed propagation

Bacuri tree seeds are oblong-angular, large, with average weight of 25.7, but with large variations that occur between genotypes and within each genotype (Table 1). The intensity of angles is conditioned by the number of seeds that form in the fruit. The face where the raphe line is situated is generally slightly concave and the opposite side is convex. In the case of seeds from the same locule of the ovary, the shape is quite irregular and dependent on the number of seeds that form in the locule (MOURÃO, BELTRATI, 1995). Seeds that originate from ovules situated in the same locule are generally lightly welded together and have a flat contact face.

Bacuri tree seeds present a particular type of dormancy, whose site of action is located in the plumule, which implies very peculiar characteristics in germination, identifying four morphological events distributed in time. The first event is represented by the emission of the primary root, which is a fast and relatively uniform process, occurring on average 18 days after sowing. Then, a phase occurs in which the primary root grows vigorously, reaching length of 180 cm, 210 days after sowing. The third event is represented by the emergence of the epicotyl, which is quite slow and with marked unevenness, occurring between 198 and 1020 days after

sowing. At that time, the primary root has average length of 186.2 cm and diameter at the basal portion of 0.71 cm. Since then, the growth of this structure is small, indicating that the rest of the reserves stored in the seed are being mobilized primarily for the growth of the epicotyl and secondary roots that although abundant are, to that moment, of small size. A fourth phase is represented by the opening of the first pair of metaphiles, which occurs, on average, 14 days after the epicotyl ruptures the integument (CARVALHO et al., 1998; CARVALHO; MÜLLER, 2007). When the plant reaches 10 to 15 cm in height, the formation of several adventitious roots at the base of the stem occurs, which function is not adequately elucidated, although Carvalho and Müller (2007) speculate that they are important for plant survival after planting at the definitive site. Adventitious roots are quite fragile when compared to primary roots. It should be noted that in other species of the Clusiaceae family, adventitious roots are common, with a difference: adventitious roots will form in the definitive root system of the plant. In mangosteen (*Garcinia mangostana* L.), for example, the emergence of a thin primary root of embryonic origin occurs at the opposite pole at which the plant stem originates. The emergence of this root is quick, and its beginnings can be observed between five and seven days after sowing. Later, on the occasion of the stem emergence, the formation of a vigorous adventitious root at its base is observed, with few secondary roots, which will be the definitive root system of the plant, since the root of embryonic origin grows only about 5 to 7 cm and then fades (CARVALHO, 2014). The same occurs in the “bacurizinho” seed (*Garcinia acuminata* Planch. & Triana; sinonímia: *Rheedea acuminata* (Ruiz & Pav.) Planch. & Triana)), that is, the emergence of a fragile root of embryonic origin followed by the formation of a robust adventitious root that originates at the base of the stem soon after the emission of this structure and that will be the definitive root system of the plant (NASCIMENTO et al., 2002).

**Table 1** - Mean, minimum and maximum values for the weight (g) of seeds of ten bacuri mother plants (*Platonia insignis* Mart.)

Mother plant	Value		
	Mean	Minimum	Maximum
CPATU 114-4	27.6 (±6.5)	15.8	45.8
CPATU 124-3	22.9 (±4.8)	15.9	34.8
CPATU 161-6	34.4 (±4.9)	24.0	47.7
CPATU 204-3	31.6 (±5.6)	20.7	44.0
CPATU 204-4	17.6 (±9.4)	4.3	35.6
CPATU 208-4	23.2 (±4.1)	15.1	35.6
CPATU 215-3	28.8 (±6.0)	18.8	48.6
CPATU 253-3	24.8 (±4.1)	17.6	32.7
CPATU 253-8	30.7 (±6.6)	13.5	43.1
CPATU 255-6	15.7 (±4.2)	7.0	23.0
Mean	25.7 (±6.1)	-	-

1. Values represent means (± standard deviation), n = 50. Source: CARVALHO, J.E.U. de e NASCIMENTO, W.M.O. do (Unpublished data).

Several pre-germination treatments applied to seeds, involving the removal of the integument, the use of growth regulating substances, in particular gibberellic acid, were not successful in terms of accelerating and standardizing epicotyl emission. The pre-treatment of seeds with growth regulating substances has as barrier the fact that bacuri seeds exhibit recalcitrant behavior in storage (CARVALHO; MÜLLER, 2007). Therefore, due to the fact that they do not withstand drying, it becomes difficult to absorb these substances, because seeds have high water content that must be maintained to ensure viability. When seeds newly extracted from fruits, when they have water content of  $39.1 \pm 3.0$ , after immersion in gibberellic acid solution for 24 hours, their water content is usually increased by only one percentage point to  $40.1 \pm 2.8\%$ , indicating that the amount of solution absorbed is insufficient for any physiological response.

As a result of the germination characteristics, the production of seedlings or rootstocks by seminiferous means is impracticable, since the time required for seeds to complete the germination process is quite long and it must be considered that after emergence of the aerial part, an additional period for growth is also required, which varies from four and six months, so that the seedling is suitable for planting at the definitive site or the rootstock in condition to be grafted.

An additional problem regarding the propagation of bacuri tree by seeds is related to the fact that when removed from the nursery, seedlings lose much of their primary root, because long before reaching the ideal planting point, the primary root has already broken the bottom of the container, being mostly under the ground. The minimum size of plastic bags for production of bacuri tree seedlings is 35 cm in height and 18 cm in width. Thus, when the seedling is removed from the nursery, more than 1.5 m of the primary root remains under the ground, being part of the seedling only the basal portion, which reaches a maximum of 30 cm in length. As a result, plant survival is often compromised, not being rare survival rates of only 60% one year after planting. It is also noteworthy that the growth of the aerial part of the plant is impaired, because in newly planted seedlings, much of photoassimilates are carried for the regeneration of the primary root.

### **Technological alternatives to overcome the problem of slow germination**

Technological alternatives for the propagation of bacuri tree by seminiferous route more quickly involve two procedures: the first consists of the use of segments of primary root of seeds at the beginning of germination as propagation structure, and the second consists of direct seeding of seeds at the definitive site.

### **Propagation of bacuri tree by means of the primary root**

In view of the difficulties of obtaining seedlings or rootstocks by seminiferous route, a propagation method that uses seeds only to obtain the primary root was developed, since this structure has high regeneration capacity. In order to obtain primary root segments, seeds must be seeded in a depth of 1.20 m, with sand and sawdust powder as substrate. These two components should be mixed at volumetric ratio of 1: 1. After 120 to 150 days of sowing, most seeds already have a primary root with a length equal to or greater than 1.10 m and are then carefully removed from the sowing substrate. The primary root is then divided into segments with length between 7 cm and 8 cm, neglecting the lower third that has reduced diameter and tender tissues, which greatly hinders regeneration. Cuts should be performed in the transverse direction, both in the apical portion and in the basal portion of the pile. Approximately ten cuttings can be obtained from each primary root. Seeds from which the primary root is detached may be sown again, allowing obtaining new primary root segments. In this case, the time required for the primary root to reach a length equal to or greater than 1.10 m is slightly higher, requiring, generally, 180 days, since it will require healing and regeneration of the primary root, from the small segment that remained attached to the seed (CARVALHO et al., 1998).

After obtaining cuttings, they are vertically arranged in plastic bags with dimensions of 18 cm in width and 35 cm in height, containing soil as substrate, preferably of sandy texture or clayey sand, previously fertilized with NPK fertilizer with formulation 14-14-14 of slow release. When placing the cuttings on the substrate contained in plastic bags, it is of great importance that the proximal portion is oriented upwards and the distal portion downwards, otherwise the seedling obtained will present abnormal conformation, that is, the root will emerge from the distal part of the cutting, and will be directed downwards due to the positive geotropism and the aerial part that will originate from the proximal portion will be directed upwards due to the negative geotropism. It should be noted that the cuttings should be arranged in plastic bags in such a way that the apical portion is at the same level as the substrate. The newly planted cuttings should remain in nursery with 50% of sunlight interception and should be irrigated in the morning and afternoon. Seedlings must remain in this condition until they are suitable for planting or to be grafted (CARVALHO et al., 2002).

In most cuttings, root regeneration precedes that of the aerial part. It is a quick process, regardless of the mother plant that gave rise to seeds, requiring, on average, 65 days, with regeneration percentage higher than 80% (Table 2). With this propagation system, it is possible to produce bacuri tree seedlings or rootstock within a year, since seedlings are suitable for planting or to be grafted between six and eight months after the placement of cuttings in the substrate. The seedlings obtained by this propagation system are, morphologically, similar to those originating from seeds, presenting a pivotal root; however, they are smaller (Figure 5).

Primary root propagation can be more easily performed by direct sowing in plastic bags, with subsequent separation of the primary root from the seed that originated it. In addition to being easier to perform, as it does not require the stage of sowing seeds in sowing containers, it allows obtaining more vigorous seedlings, since the primary root segments are larger. The procedures to obtain seedlings by direct seeding of seeds in plastic bags with subsequent separation of the primary root, established by Carvalho and Müller (2007) are composed of the following steps:

**Table 2** - Percentage and mean time required for regeneration of bacuri plants (*Platonia insignis* Mart.) from primary root segments of seeds originating from ten mother plants.

Mother plant	Regeneration (%) <sup>1</sup>	Mean time for regeneration (day)
C1	82.5 (±9.6)	61.0 (±4.8)
C2	82.5 (±9.6)	78.5 (±5.1)
C3	95.0 (±5.8)	51.6 (±3.4)
C4	95.0 (±5.8)	73.0 (±6.6)
C5	85.0 (±2.9)	56.8 (±5.0)
C6	82.5 (±5.0)	74.9 (±7.8)
C7	87.5 (±5.0)	64.3 (±5.8)
C8	77.5 (±9.6)	58.4 (±6.3)
C9	80.5 (±8.2)	66.4 (±5.4)
C10	75.5 (±5.8)	59.7 (±5.4)
Mean	84.4 (±6.6)	64.5 (±8.7)

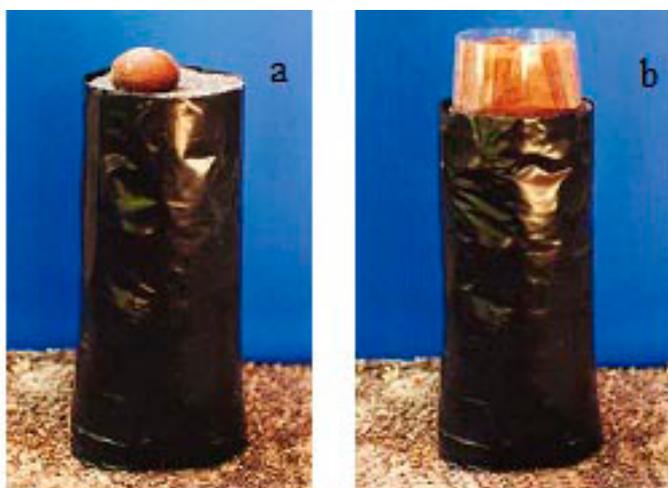
1. Values represent means (± standard deviation), n = 4 repetitions of ten cuttings. Source: CARVALHO, J.E.U. de e NASCIMENTO, W.M.O. do (Unpublished data).



**Figure 5** - Bacuri plants (*Platonia insignis* Mart.) originating from primary root cuttings.

a) Sowing must be done in plastic bags with minimum dimensions of 18 cm in width, 35 cm in height and 200  $\mu$  in thickness, containing as a substrate the mixture of 60% soil, 20% sawdust powder and 20% tanned manure or, alternatively, 60% soil and 40% aviary bed. Bags should be completely filled with this mixture and the seed placed on the substrate so that the point from which the primary root will emerge approximately coincides with the center of the container (Figure 6a). Subsequently, a protective cylinder is placed around the seed. This cylinder can be made of plastic or aluminum, with height between 7 cm and 8 cm and diameter between 10 cm and 11 cm and must be filled with sawdust or vermiculite, completely covering the seed (Figures 6b). PET bottles with capacity of at least two liters, can be used to make these cylinders.

b) Between 70 and 100 days after sowing, when the primary root of almost all seeds has already reached the bottom of the plastic bag, the cylinder is removed and the substrate is removed in such a way as to expose the seed and the basal portion of the primary root (Figure 7a). Then, the primary root is separated from the seed that originated it, with a cross-section cut with a pocketknife at a distance of 0.5 cm to 1.0 cm from the seed (Figure 7b). The function of the cylinder is solely to facilitate the operation of separating the primary root segment from the seed that originated it, since its non-use would entail removing part of the substrate from the plastic bag, which is much more difficult and time-consuming.



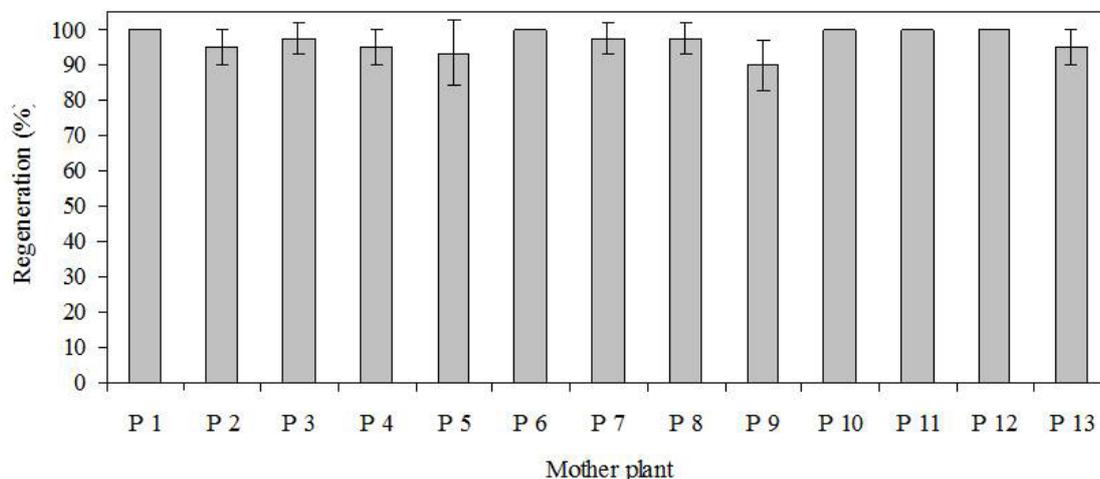
**Figure 6** - Seed arrangement during sowing, with the root pole facing the center of the container (a) and seed protection with plastic cylinder filled with sawdust (b).



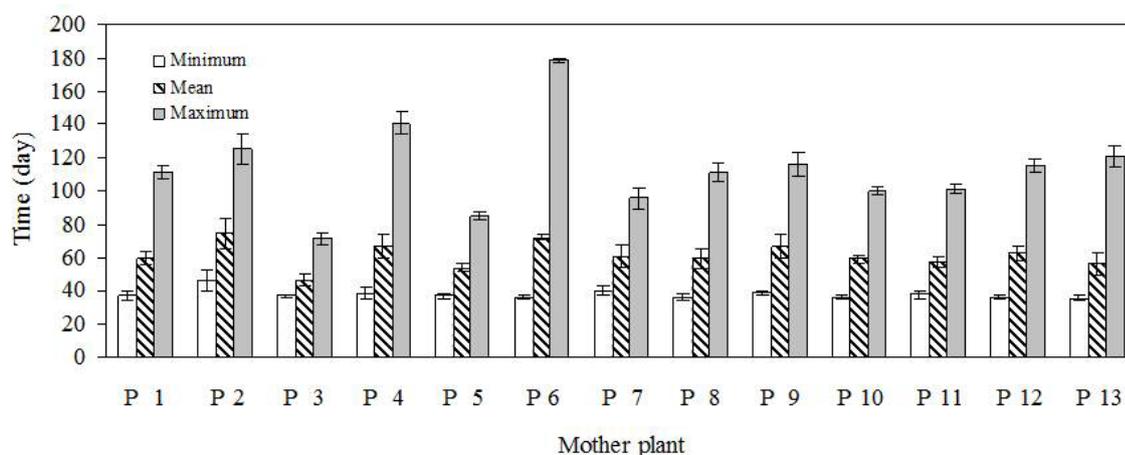
**Figure 7** - Removal of the cylinder (a) and separation of the primary root from the seed that originated it (b).

The regeneration percentage, regardless of the genotype of the mother plant, is high, with values higher than 90% and it is processed in a relatively fast time, starting between 35 and 46 days and finishing between 71 and 179 days after the planting of cuttings. On average, 61.2 days are required for plant regeneration (Figures 8 and 9).

From a seed, it is possible to obtain three to four seedlings, and in the third or fourth, the epicotyl is of embryonic origin. For this, it is necessary that immediately after the separation of the primary root, seeds are immediately sown, and all procedures are repeated (CARVALHO et al., 1998).



**Figure 8** - Regeneration percentage of bacuri plants (*Platonia insignis* Mart.) originating from the primary root of seeds of different mother plants. Source: CARVALHO, J.E.U. de; NASCIMENTO, W.M.O. do. (Unpublished data).



**Figure 9** - Minimum, mean and maximum times required for the regeneration of bacuri plants (*Platonia insignis* Mart.) from the primary root of seeds from different mother plants. Source: CARVALHO, J.E.U. de; NASCIMENTO, W.M.O. do. (Unpublished data).

It should be emphasized that when the purpose of planting is the production of fruits, propagation by primary root segments of seeds at the beginning of germination is particularly indicated to obtain rootstocks, since the bacuri tree is an essentially allogamous species, and the alogamy rate is determined by genetic self-incompatibility mechanism. In addition, plants propagated only start fruit production between ten and twelve years after planting.

#### Propagation by direct sowing of seeds in the field

This propagation system was recently developed and successfully tested in the Brazilian Eastern Amazon, in areas of Af and Am climatic types, according to Köppen classification. It involves simple and low-cost procedures, since there is no need for irrigation, even in the period of lower rainfall, and costs are fundamentally represented by the control of weeds in a radius of 30 cm from the pit.

**Table 3** - Emergence percentage, height, basal diameter and number of leaves of bacuri trees (*Platonia insignis* Mart.) one year after direct seeding in the field.

Plot	Emergence (%)	Height (cm)	Basal diameter (cm)	Number of leaves
1	60.0	41.7	0.7	15.0
2	58.0	30.9	0.5	11.9
3	46.0	22.7	0.5	8.5
4	66.0	22.5	0.5	9.9
5	44.0	28.2	0.5	10.2
Mean <sup>1</sup> (±standard deviation)	54.8 (±8.4)	29.2 (±7.9)	0.52 (±0.10)	11.1 (±2.50)

1. Values represent averages (± standard deviation), n = 5 replicates of 50 seeds. Source: CARVALHO, J.E.U. de; NASCIMENTO, W.M.O. do. (Unpublished data).

It basically consists of the opening of a small hole of 12 cm in diameter and 8 cm in depth in which at least three seeds are sown. The recommendation of three seeds per pit is to ensure that, in one year after sowing, in each pit at least one seed has already germinated, since the emergence percentage after that period is between 44% and 66%. At that time, plants had mean height of 29.2 cm, basal diameter of 0.52 cm and 11 leaves (Table 3). The great advantage of direct seeding is that the primary root is kept intact, with faster growth of plants, so that between 14 and 18 months after sowing they are already able to be grafted, since they are taller than 60 cm and have basal diameter between 1.5 cm and 4.0 cm. In addition, when grafted, grafts grow more vigorously when compared to growth of seedlings grown in containers. It is also noteworthy the fact that grafting can be done directly in the field, both by the full cleft grafting and budding methods, as will be presented in the topic on which asexual propagation is addressed.

In order to be successful in direct seeding, it is essential that the seeds be sown at the appropriate time, that is, during the period when the rainy season is consolidated. In the Brazilian Eastern Amazon, this period involves the months of February and March, when the availability of bacuri seeds is also great, as in these months, peak fruit production occurs. Seeding in February and March is important so that the availability of water does not constitute a limiting factor for the emergence and growth of the primary root. In addition, it is of considerable interest that when the period of lower rainfall is approaching, the primary root is longer than one meter, which ensures the continuity of the germination process, since roots are already in depth at which soil moisture remains at a relatively stable level.

#### Asexual propagation of bacuri tree

Despite the fact that bacuri tree in nature reproduces easily by shoots from roots, the removal of these shoots to the production of seedlings, in the current state of knowledge, is not an efficient propagation method, since

the survival rate of these shoots in nursery is very low and does not reach 25% (LIMA, 2000). In addition, plants from shoots present, in the first six years, a root system with only one lateral root that grows quite considerably in length. This makes the plant quite susceptible to bending by the action of winds (Figure 10). Thus, plants originating from shoots of roots have to be tutored with resistant stations of 2.5 m in length, which must be buried at 0.5 m. Between the sixth and the eighth year after planting, new lateral roots that develop vertical roots emerge, which reach more than 2 m of depth, guaranteeing plant sustentation.

Propagation by root cuttings of adult plants is also difficult. These cuttings easily emit aerial part in a period of less than 65 days, but fail to emit roots, even with the use of substances that induce rooting (CARVALHO; MÜLLER, 2007).

#### Propagation by grafting

The conventional grafting method of bacuri tree involves the formation of the rootstock that is usually the bacuri tree itself obtained by seeds or by any of the previously described methods. Cleft grafting, besides being a method of easier execution and with higher labor yield, provides higher percentage of viable grafts than spliced side grafting. In both methods, budding of grafts begins 20 days after grafting, but can last up to 80 days, when the percentage of grafts sprouted reaches 80% and 42%, respectively, for top and spliced side grafting, respectively (CARVALHO et al., 2002).

The grafting success depends, among other factors, on the time of removal of the tips and their diameter. Usually, higher percentage of viable grafts is obtained when tips are removed prior to the full exchange of the leaves of the mother plant to be propagated. Usually, in the period from November to May, tips are in an ideal stage to be grafted, with mature leaves, woody tissues and apical bud still in the dormancy phase. When tips from plant that are in the phase of renewal of leaves or very close to that phase are used, the budding of grafts occurs in a short period of time, even before the welding with the rootstock

and almost all grafts die (CARVALHO; MÜLLER, 2007).

For grafting carried out on rootstocks, the wet chamber consists of a transparent plastic bag internally moistened with water. In the case of grafting carried out on rootstocks from direct seeding, better results have been obtained when grafting is involved with a layer of newspaper coated with aluminum foil and with an outer layer of newspaper, as it provides higher percentage of sprouted grafts. The use of a wet chamber consisting of a plastic bag internally moistened with water covered with newspaper also results in good percentage of sprouted grafts. On the other hand, the traditional wet chamber used for nursery-grafted plants is not adequate, leading to low percentage of sprouted grafts due to the greenhouse effect of the plastic bag directly exposed to sunlight (Table 4).

With regard to the propagation of bacuri tree by the budding method, the results are still very incipient. Even so, some plants of the Embrapa Amazônia Oriental Germplasm Active Bank have already been propagated by this method, which in relation to the previous one has the advantage of not having to protect the graft with wet chamber, which is of considerable interest, particularly when rootstocks come from direct sowing. This grafting method, although providing high percentage of live grafts, generally superior to 85%, the sprouting of grafts is delayed and uneven, requiring periods of more than three months to obtain percentage of sprouted grafts greater than 80%. The causes of this delay in sprouting need to be investigated. Although it is possible to speculate that they are associated with the dormancy of buds, this hypothesis does not find support since it has been observed differential behavior of buds of the same stem in terms of time required for graft sprouting.

### ***In vitro* propagation**

At present, there are no established protocols for the propagation of bacuri tree by means of tissue culture. The few studies previously developed have addressed only aspects concerning the decontamination of explants (FERREIRA et al., 2009; SANTOS et al., 2015).

### **Concluding remarks**

The systems of production of açai tree seedlings by sexual route are already well established. The technologies so far developed allowed producing quality seedlings from

seeds, with excellent survival rate after planting and good growth of plants in the field. The great challenge involves the development of technology that allows for the large-scale propagation of species by asexual route, both from tiller and somatic embryogenesis.

With regard to bacuri tree, despite the few researches developed so far, considerable advances in both sexual propagation and asexual propagation have been obtained in recent times. It is noteworthy that until the end of the 20<sup>th</sup> century, the time required to produce a bacuri tree seedling by sexual route was at least two and a half years and with the technologies developed, from then on, it is possible to produce grafted seedlings within one year. It is of considerable interest that these works continue to further improve the seedling production systems of the species. In addition, it is important to test alternative rootstocks of similar species, since the genus to which the bacuri tree belongs is monotype. Among these species, “bacurizinho” (*Garcinia acuminata* Planch. & Triana), “bacurizinho liso” (*Garcinia brasiliensis* Mart.), “bacupari” (*Garcinia gardneriana* (Planch. & Triana) Zappi), “bacuripari” (*Garcinia macrophylla* Mart.) and “bacuri-de-anta” (*Moronobea coccinea* Aubl.) can be mentioned, all native to the Amazon. It should be noted that at Embrapa Amazônia Oriental, some bacuri tree genotypes have already been successfully grafted on species of the genus *Garcinia*. However, the behavior of plants at field level has not been evaluated.

**Table 4** - Sprouting percentage of bacuri trees (*Platonia insignis* Mart.) directly grafted into the field by the cleft grafting method with three types of wet chamber.

Graft protection	Sprouted grafts (%)
Newspaper coated with aluminum foil and newspaper	87.5 <sup>1</sup> (±9.5)
Plastic bag coated with newspaper	75.0(±10.0)
Plastic bag	47.5(±5.0)

1. Values represent averages ( $\pm$  standard deviation), n = 4 replicates of 10 grafts. CARVALHO, J.E.U. de; NASCIMENTO, W.M.O. do. (Unpublished data).



**Figure 10** - Bacuri tree (*Platonia insignis* Mart.) with four years of age, originated from root shoots, bent by the action of winds.

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