PN-AAY-581

AGENCY FOR INTERNATIONAL DEVELOPMENT
WASHINGTON, D.C. 20523

DATE: 10/22/87

MEMORANDUM

TO: AID/PPC/CDIE/DI, room 209 SA-18

FROM: AID/SCI, Victoria Ose VO

SUBJECT: Transmittal of AID/SCI Progress Report(s)

Attached for permanent retention/proper disposition is the following:

AID/SCI Progress Report No. 1.A-02
rec'd 10/22/87

Attachment

2 cys

1. A- 02

Report on Studies of Babassu Palms (Orbignya phalerata)

Undertaken in Brazil

A US-Brazil Collaborative Research Program Sponsored

By the U.S. Agency for International Development

Grant No. DAN-5542-G-55-1089

("Domestication of the Babassu Palm")

Rec'd in SDI: OCT 22 1987

INTRODUCTION

In 1981 The New York Botanical Garden received a grant from the Science Advisor's Office of the U.S. Agency for International Development (#DAN-5542-G-55-1089), "Domestication of the Babassu Palm." This research was a collaborative effort between a number of agencies in the U.S. and abroad, primarily in Brazil. The Brazilian counterpart agency, CENARGEN (Centro Nacional de Recursos Geneticos), was responsible for coordinating research efforts on babassu palms carried out under this program in that country. The following report is divided into three sections, conforming to the research objectives stated in the original proposal: Germination of Babassu Kernels; Germination of Babassu Embryos *in vitro*; and, Conservation of Babassu Germplasm. This report discusses the period of the grant, 1981-1986.

Michael J. Balick
The New York Botanical Garden
Bronx, New York 10458 USA

1 July 1987

CONSERVATION OF BABASSU GERMPLASM

José Mario Ferro Frazão
Claudio Urbano Bittencourt Pinheiro

Babassu, as is the case with most palms, is a recalcitrant species. In other words, it is impossible to maintain seeds for a prolonged period in conservation chambers, making it necessary to maintain the germplasm in the field.

Conservation of germplasm in the field involves much more work and is generally much more expensive than conservation of seed. Critical aspects involved are the maintainance of germplasm for indefinite periods in the field, usually occupying large areas, and equally or more important, guaranteeing that the germplasm is representative of the genetic variability found in the populations sampled.

These two parameters make it very important to establish a reliable routine for the treatment of the germplasm collected so that loss of material is kept to a minimum.

As in most tropical species, little was known on the biology of babassu at the start of this project, and even less data was available on techniques and problems involved in the adequate conservation of germplasm for the species involved.

The basic routine here described for babassu was developed during 1981/1982 for the germplasm collected in 1981,

and has been used to present with minor modifications.

Figure 1 shows the flow of activities involved with the introduction and conservation in the two babassu gene banks throughout the year. Some of the items, such as germplasm collection and kernel germination are detailed elsewhere and will only be treated briefly here.

Five basic phases or stages have been considered:

- Research, which underlies all activities carried out in the gene banks;
- Nuts, including all activities involving whole babassu nuts;
- Kernels, encompassing all work done with kernels;
- Seedlings, which includes the handling and treatment at the seedling stage, and
- Field, including activities involved with final establishment of the germplasm in the gene banks.

The whole cycle, from collection to establishment in the field takes almost two years, as can be observed in the flow chort.

Germplasm Collection

This item has been fully discussed elsewhere. Here it is sufficient to note that twenty individuals were sampled per population and that it was considered that for an adequate sample of the population, 12 scions of each individual should be

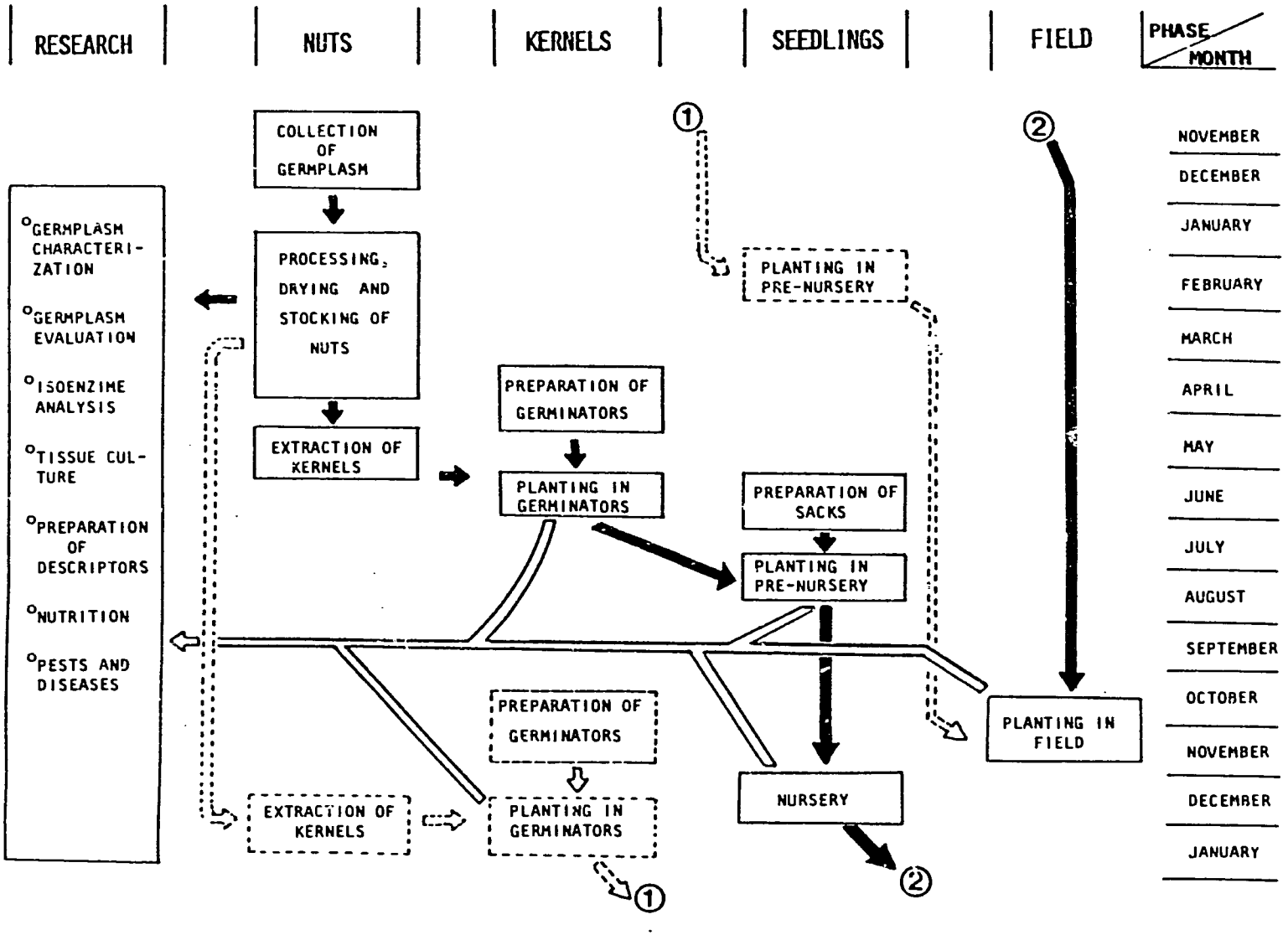


Figure 1. Flow of activities involved in the conservation of babassu germplasm. Black: flow of material; open: flow of research data; dotted: alternate flow of material (repo sition).

represented in the collection, giving a total of 240 plants per population.

Processing, Drying and Stocking of Nuts

Material collected is registered,cleaned and fumigated. Fruits are then dried by exposure in the sun for approximately two weeks, and then stocked in a cool, dry room.

Subsamples are separated for research purposes (isoenzyme analysis, tissue culture and phenotypic characterization).

Extraction of Kernels

As mentioned elsewhere, germination of whole fruit has proven to be unreliable. It was therefore necessary to work with individual kernels. Kernel extraction was done by the traditional hand-cracking of the fruit, which has been amply described elsewhere. Special care was taken to obtain undamaged kernels.

Planting in Pre-nursery

The pre-nurseries consist of large open sheds with capacity for approximately 1500 sacked plants each. Construction materials used were almost wholly devided from babassu, with babassu thatching used for shading.

Germinated kernels are transfered to 40 x 50 cm

poliethylene bags filled with a mixture of 3 parts soil to 1 part cured cattle manure. Bagged plants are maintained separated by populations and matrixes with virtually no spacing between individuals derived from the same matrix (parent plant).

Seedlings are maintained under shade in the pre-nursery until the emergence of the 4th leaflet (ca. 180 days).

It was observed that in the pre-nursery, root growth is significantly faster than leaflet formation, with a mass of roots rapidly occupying all the space in the sacks.

Although most of the material was healthy and growing in a satisfactory manner, some problems were noted. Many plants presented burnt leaf margins and apexes. Some were initially clorotic, but recuperated after some time.

The mayor problems faced, which affected a small percentage of the seedlings, were "damp off", with rotting of tissue at the insertion of the root with the stem, and insect attack, in which the seedlings were killed when the stem was separated from the roots.

The insect responsible for this problem is probably the larval stage of a beetle, which is fairly common in the sacks.

Although it was initially considered that soil sterilization might be advisable, the low indexes of insect attack as well as rot by "damp off" were not considered to be sufficiently serious to justify the great amount of extra work

involved.

Nursery

Seedlings are transfered to the nursery as soon as convenient after the emergence of the fourth or fifth leaflets. The critical factor is not the stage of the seedlings, but meteorological conditions in the region.

The plants cannot be transfered to the nursery during the dry season, and the wet season must be well established before plants are left in the open.

In the nursery, bagged plants are placed in the open with a spacing of 1.0 x 1.5 m for hardening for field conditions. Plants remain in the nursery until ca. 8 leaves have emerged and a strong root system is completely formed (approximately 330 days).

Planting in the Field

At present, each of the two babassu germplasm banks has an available area of 50 hectares for the establishment of the permanent collection. Based on experience with other palms, spacing in the field is 6 x 7 m (Bacabal) or 8 x 8 m (Teresina) giving a total of 240 or 157 plants respectively.

Land is cleared as needed, using a bulldozer, with manual clearing of roots and other residue. After clearing, it is graded, and 50 x 50 x 50 cm holes dug at the spacings cited above.

Within each population, individuals are planted in a

random distribution to permit reliable characterization and evaluation in the gene bank. Each individual is planted in a mixture of soil and 2 kgs of dried manure.

As in the nursery, planting time is of critical importance, and must be done at the beginning of the rainy season.

Reposition of Germplasm

In spite of the relatively high survival rates in all the different stages, a certain amount of material is lost in each phase, making it necessary to replace this material.

When the germplasm is in the pre-nursery, it is usually possible to determine the amount of material that will be necessary to replace that which has been lost. An alternative flow (dotted lines in diagram) is necessary, using nuts that have been stored as a technical reserve.

Conservation Units

In 1981, a first babassu gene bank was established at EMAPA facilities in Bacabal, Maranhão in a collaborative effort between INEB, EMBRAPA(CENARGEN and UEPAE-Teresina), EMAPA and The New York Botanical Garden with financial support from US-AID. Due to UEPAE-Teresina's involvement as coordinating Institution for The National Babassu Program, a second babassu gene bank was established in 1984 at UEPAE-Teresina in Teresina, Piauí in collaboration with CENARGEN.

Babassu Gene Bank at Bacabal

Location: Bacabal, Maranhão, Brazil

Latitude: 04°14'S

Longitude: 44°43'W

Altitude: 38 m above sea level

Mean yearly temperature: 26.4°C

Mean yearly rainfall: 2007 mm

Total area available: 50 hectares

Area presently planted: 6 hectares

Number of accessions: 95

Number of populations represented: 6

Person responsible: Claudio Urbano Bittencourt Pinheiro

The Bacabal gene bank was established in November 1981, and by May 1982 all basic facilities (germinator, prenursery, nursery, storage facilities and well) had been installed.

Introduction of germplasm to the gene bank was initiated in January 1982 with the material collected in the first international expedition funded by US-AID through the New York Botanical Garden.

Preliminary experiments in kernel germination were carried out in February/March of the same year, and routinely established from April onwards.

Although germination techiniques were well avanced, it was found that germination was not uniform for all the six populations tested. As all of the material had been collected

during the same expedition, and since subsequent attempts at replication showed essentially the same results, it was concluded that there is a significant difference in viable seeds within and between populations, and further research is needed in this matter.

In December 1982 and January 1983, a six hectare plot was cleared for establishment of material in the field, and seedlings were planted in a 6 x 7 m spacing scheme.

Although there has been some problem with excess rainfall and flooding in 1983 and 1984, the condition of the material in the gene bank can be considered as satisfactory.

Table 1 lists all material presently found in the Bacabal gene bank.

Babassu Gene Bank at Teresina

Location: Teresina, Piauí, Brazil

Latitude: 05°05'S

Longitude: 42°48'S

Altitude: 72 m above sea level

Mean yearly temperature: 27.4°C

Mean annual rainfall: 1297 mm

Area available: 50 hectares

Area presently planted: 1,5 hectares

Number of accessions: 95

Number of populations represented: 12

Person responsible: José Mário Ferro Frazão

The gene bank at Teresina was established in October, 1984, and at the beginning of 1985 all basic facilities had been installed.

From February of 1985 to present germplasm representing 12 of the populations sampled since the beginning of the project have been introduced. Some of the material is already in the field, while germplasm collected in later expeditions is presently in different stages of the process.

To present, all the germplasm accessed is in excellent condition.

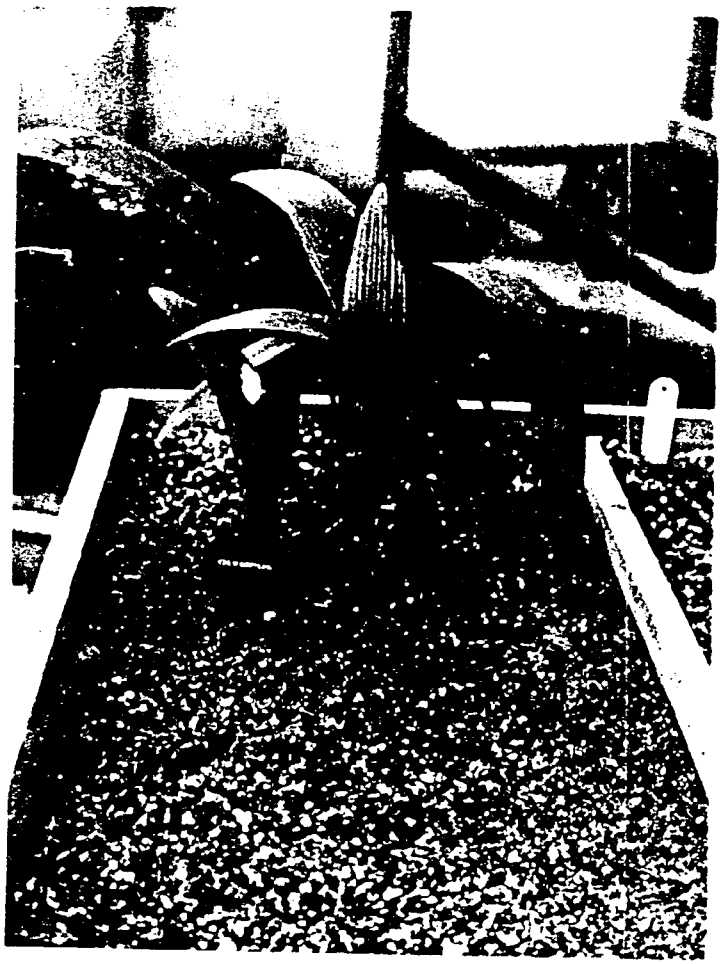
Table 2 lists all material presently accessed in the Teresina gene bank.

Figures 2-9 illustrate some aspects of the babassu gene banks at Bacabal and Teresina.

A

B

Figure 2. Some of the instalations at the gene bank in Bacabal, November, 1982. A and B: Foreground, storage shed; background, pre-nursery.



A

B

Figure 3. A: view of a germinator at UEPAE-Teresina, 1985. Note that kernels are maintained in germinator until emergence of first leaf. B: Plants in pre-nursery at Bacabal, November, 1982.

A

B

Figure 4. A and B: Pre-nursery at Bacabal, November, 1982. Note babassu thatching used. Plants arranged by population and by matrix.

A

B

Figure 5. Some phytosanitary problems found in the gene banks. A: rot affecting kernels in germinator, probably due to injury in kernel extraction; B: decapitation of seedling, probably caused by a beetle.

A B

Figure 6. Some phenotypic variations observed in the pre-nursery. A: clorosis; B: variegated nervations. Both of these disappear in subsequent stages.

A

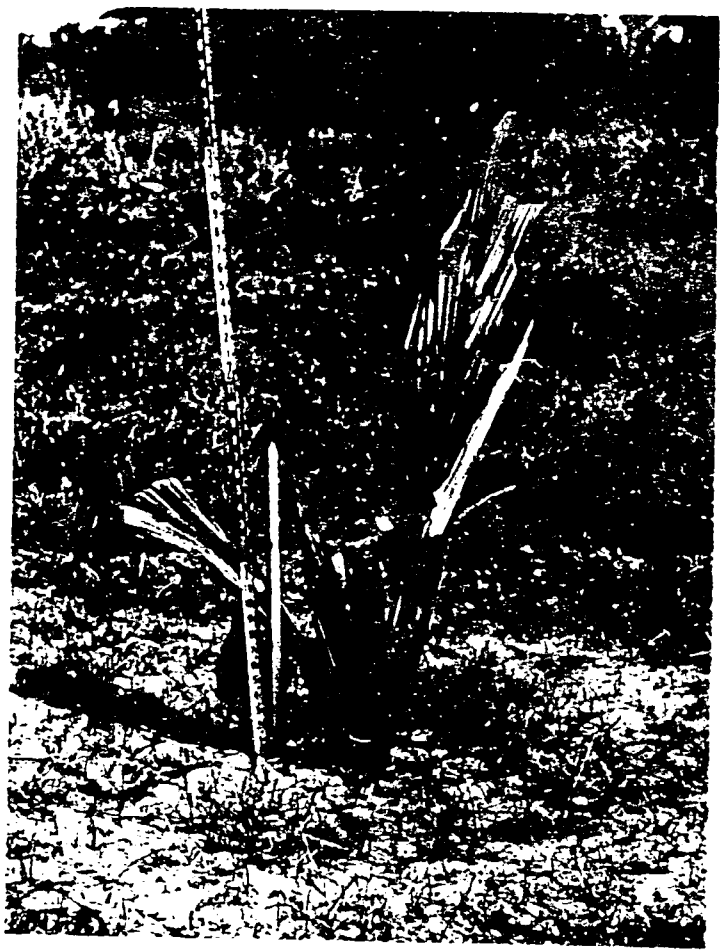
B

Figure 7. A and B, seedlings in nursery at UEPAE-Teresina, 1985, just prior to removal of shading. Note spacing.

A

B

Figure 8. A and B, views of permanent collection at UEPAE-Teresina, 1985, shortly after planting of material in field.



A

B

Figure 9. A and B, seedlings in permanent collection ate UEPAE-Teresina, 1985, shortly after transfer from nursery.

GERMINATION OF BABASSU KERNELS

José Mário Ferro Frazão

Claudio Urbano Bittencourt Pinheiro

Reliable whole fruit germination is one of the most vexing problems faced when working with palms. With the exception of a few species where germination is not difficult, and where techniques have been mastered, such as the peach palm (Bactris gassipaes) some species of Euterpe (i.e. E. oleracea), the Jessenia/Oenocarpus complex and a few others, most palms are remarkably difficult to germinate, and usually take a long time between planting and germination. Over the years, several articles have touched on the subject (i.e. Koebernik, 1971), and recently Pinheiro (1984) has reviewed the literature on the same. Methods proposed have become increasingly more complex, without, however, becoming significantly more dependable.

In babassu, trails carried out between 1978 and 1980 clearly indicated that whole fruit germination was not satisfactory for research purposes. Fruit germination took between 70 days and a year, with a success rate of ca. 15%. Lack of uniformity in germination time also constituted a serious drawback to whole fruit germination, as it made impossible to establish uniform field experiments.

With approval of this project by US-AID, it become imperative to develop reliable methods that would permit the conservation of the babassu germplasm collected, as well as to permit the establishment of homogeneous material for field trails.

One of the most attractive alternatives to whole fruit germination is the germination of individual kernels. In addition to multiplying the number of plants that can be obtained per fruit, the elimination of the woody endocarp should greatly simplify germination.

Although initial tests were initiated slightly before formal collecting expeditions, most of the methods were developed and perfected on the germplasm collected during the US-AID sponsored field trip carried out in late 1981.

METHODS

A open-sided germination shed was built, using babassu as the main construction material.

Several dozen marine plywood germinators 40 x 50 x 10 cm were built.

Kernels were obtained through the time-honored methods of manual cracking of the fruit. For this purpose, an experienced local person was hired. Special care was taken to avoid damage to the kernels during extraction, and undamaged kernels were selected for planting.

initial trails used sterile sand as a substrate, which was later substituted for vermiculite. Effects of giberelic acid and a fungicide, Thiuran, were also tested.

- Treatments

Giberelic acid (GA_3) was used at a concentration of 0,3%, with immersion of the kernels for 3 hours. Thiuran was applied to the kernels as 1% dry powder.

Treatments tested were the following:

- Control (no treatment)
- Giberelic acid
- Fungicide
- Giberelic acid + Fungicide

In sand, 50 kernels per treatment were tested, while 40 kernels per treatment were used in vermiculite.

Table 1 presents results obtained with the different treatments using the two substrates. As can be observed from the results, sand was not found to be satisfactory as a substrate. Giberelic acid enhanced germination, while treatment with fungicide alone had an inhibitory effect. Treatment with both GA_3 and fungicide proved to be the most effective.

TABLE 1. Babassu kernel germination with different treatments using two substrates. (1). Live and dead kernels are based on examination of embryos at end of experiments.

SUBSTRATE	TREATMENT	LIVE KERNELS, NOT GERMINATED	DEAD KERNELS (1)	KERNELS GERMINATED	% GERMINATION
SAND	CONTROL	33	12	05	10
	GA_3	28	16	06	12
	THIURAN	45	05	00	-
	GA_3 + THIURAN	30	13	07	14
VERMICULITE	CONTROL	25	12	03	10
	GA_3	16	09	15	37,5
	THIURAN	36	04	00	-
	GA_3 + THIURAN	07	16	17	42,5

Germination was found to be very uneven and took up to thirty days. Both mecanical and manual scarification of the germination pores of the kernels was then tested to see the effects on germination. Mecanical scarification did not prove to be satisfactory, as many of the kernels were harmed. Manual scarification has reduced germination time to 3 days with ca. 90% germination rates.

In the past, kernels were considered germinated when the conduct (haustorium) had emerged, and attained sizes of between 2 - 3 cms, when kernels were transfered to the pre-nursery. Presently, larger germinators (40 x 40 x 20 cm) have been introduced, and the material is maintained in the germinators until the first leaf has emerged. This has simplified seedling production, reducing the risk of loss of germplasm in the pre-nursery. Costs have also been considerably reduced.

Literature cited

Koebernik, J. 1971. Germination of palm seed. Principes: 15: 134 - 137.

Pinheiro, C.U.B. 1984. Germinação de sementes de palmeiras: uma revisão bibliográfica. Typescript. 107 p.

GERMINATION OF BABASSU EMBRYOS *IN VITRO*

Isabel Curiã Cabral

Due to the difficulty in obtaining seed as well as kernel germination in Brasília, and the need for reliable material for isoenzyme analysis, it was decided to try to induce germination of babassu embryos *in vitro*.

METHODS

Fruit subsamples from the populations collected during the project were sent to Brasília for isoenzyme research.

Embryos were obtained by sawing the fruit in half and collecting the parts of the kernels that contained the embryos.

Exsiced embryos were surface sterilized by immersion during 5 minutes in a 20% solution of a comercial bleach (Q-Boa) containing sodium hipoclorite, with subsequent washing in sterile distiled water.

Embryos were planted in 100 ml flasks containing Murashige - Skoog macro and micro nutrients with 1% activated charcoal, and dextrose as an energy source. Explants were maintained in the dark at between 30 and 32°C for approximately

30 days until they were sufficiently grown to be transfered to test tubes.

After transfer to the test tubes, the plantlets were maintained in the dark until the production of third leaflet, as it was found that if they were exposed to the light before this stage, hardening of the first two leaflets difficulted the emergence of latter leaflets. Root formation was observed to occur after the emergence of the fourth or fifth leaflets.

Figure 1 shows the different stages of plantlet development in tissue culture.

RESULTS

Table 1 summarizes the results obtained with tissue culture of excised babassu embryos. As can be observed, there was a 50% survivorship of plantlets in relation to embryos planted. A contamination rate of ca 33% was observed, while approximately 18% of all embryos planted proved to be inviable.

The material used for isoenzyme analysis consisted of the haustoria of the plantlets which were excised prior to the transfer from vials to test tubes. This may explain the high indexes of mortality found after this stage (ca 29% of all material transfered).

TABLE 1. Results of *in vitro* culture of excised babassu embryos (1) Plantlets actually transfered from flasks to test tubes; (2) plantlets alive at end of experiment.

ORIGIN	POPULATION (collector's No)	INDIVIDUALS SAMPLED	EMBRYOS	PLANTLETS FORMED (1)	PLANTLETS SURVIVED (2)
PARÁ	1304	20	244	122	60
GOIÁS	1309	21	536	191	130
MARANHÃO	1342	20	463	413	240
PIAUÍ	1351	20	354	194	148
CEARÁ	1353	20	430	255	205
CEARÁ	1354	20	475	440	363
BOLÍVIA	1392	10	273	13	12
TOTAL	-	131	2275	1628	1158

Since the purpose of the research was to obtain material for isoenzyme analysis, no attempts were made to harden the plantlets for future planting in the greenhouse or under field conditions.

Figure 1. presents different stages in the process.

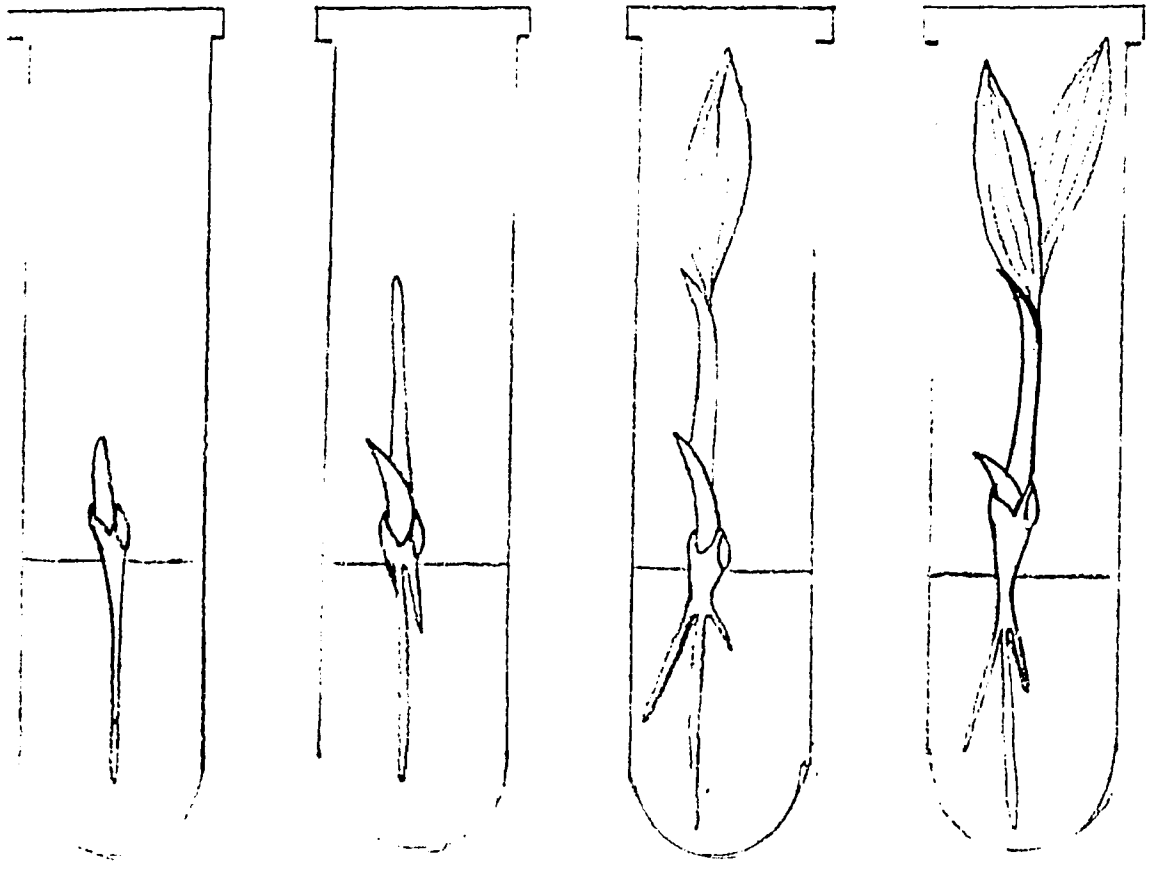


FIG 1