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Studies on the Practicality of ex situ Preservation of Palm Seeds

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Palms are among the most economically important plant families throughout their extensive distribution in the tropics and subtropics. There are hundreds of palm species of economic utility, including the familiar examples such as coconut and African oil palm, that are currently so important in international commerce (Balick and Beck 1990).

Many of these species are little known outside their natural distributions but account for a substantial economic trade. For example, in 1979 Brazil reported over $100,000,000 of commerce derived from the harvest and sale of oil, wax, beverages, foods, fibers, charcoal, and other commodities derived from six genera of native palms: *Astrocaryum*, *Attalea*, *Copernicia*, *Euterpe*, *Mauritia*, and *Orbignya* (Balick 1985). Not included in this economic survey was the impact of palm products at the subsistence level, for example, the use of palm leaves as thatch for homebuilding. If a subsistence farmer is unable to find an appropriate thatch for his house, zinc or other similar roofing must be purchased at a substantially greater cost. Although current studies attempting to place values on these substitute products are under way in the Neotropics, there is little doubt that the total value of palm products for subsistence activities far exceeds the value of the commodities that enter the economic system. Thus, intense pressure on native palm resources, as well as habitat destruction, is resulting in a massive reduction in the genetic diversity of this plant family.

The storage of seeds at low (air dry) moisture content and at low (sub-zero) temperatures offers a convenient and cost-effective means of conserving plant genetic resources in the medium and long term. It is the basis for the operation of a number of seed banks worldwide, in which useful seed storage lives are expected to be on the order of at least many tens and probably many hundreds of years (Roberts 1989). Success of this technology depends upon the ability of seeds to withstand drying to quite low moisture contents (<10% on a fresh weight basis). Low seed moisture content not only removes the possibility of damage due to freezing injury in subsequent deep-freeze (−20° C) or cryogenic (e.g., liquid nitrogen, −196° C) storage, but also itself promotes, along with reduced temperature, increased longevity in orthodox seeds (Roberts 1973).

The conservation and effective utilization of palm germplasm is beset by the same problems facing other tropical families. *In vivo* collections, both in the wild and in repositories, are subject to diseases, bud mutations, human encroachment, and poor management, as well as being expensive in terms of management inputs and space. The application of *in vitro* methods of *ex situ* conservation has several associated technical problems, including the

relatively frequent need for subculture and the fact that a single protocol is unlikely to be useful for diverse genotypes (Towill and Roos 1989).

In many areas of the Neotropics, entire palm populations have been destroyed; significant threats exist at the species level (e.g., *Attalea crassispatha*, see Henderson and Aubry 1989). If these palm resources are to be preserved for utilization and enjoyment by future generations, an increased level of action is needed in the conservation arena. For example, Brazil has a program to preserve the germplasm of economically important native palms, both *in situ* and *ex situ* (Coradin and Lleras 1988). This paper explores the possibility of *ex situ* conservation of members of the palm family in seed banks.

One group of plants, including several important tropical plantation species, bears seeds classified as recalcitrant (Roberts 1973). The main characteristic of such seeds is their inability to withstand any degree of desiccation and so they cannot be kept at sub-zero temperatures. Their lifespans are relatively short and cannot be extended by the methods presently employed to preserve germplasm in seedbanks or cryogenic stores.

Many palm species are propagated primarily by seed (Broschat and Donselman 1988) and, although Corner (1966, p. 183) maintains that palm seeds in general cannot withstand any degree of drying, the seeds of several species (e.g., date palm) are amenable to storage at freezing temperatures in the air-dry state (Al-Madeni and Tisserat 1986). However, there are also examples, such as coconut, of recalcitrant seed behavior among the Palmae (Chin and Roberts 1980), as well as at least one instance of apparently recalcitrant seeds (oil palm) being subsequently shown to be desiccation tolerant under certain conditions (Grout et al. 1983, Ellis et al. 1991). This paper reports on a study aimed at classifying seed storage behavior in a wide range of palm species, mostly having little or no previous work reported on them, with the aim of obtaining a reliable estimate of the proportion having seeds amenable to seed-banking or cryogenic storage.

Materials and Methods

Seeds of fourteen palm species were collected from sites in the United States, Central and South America, and Southeast Asia and sent to Wakehurst Place for investigation. The opportunistic nature of the collections meant that samples were often low in numbers of seeds, and the great distance they were transported meant that sometimes seeds were in poor condition on arrival at Wakehurst Place. Thus, the difficulties (including sporadic fruiting, obtaining export permits, and the time taken to transport seeds to the laboratory) inherent in working with tropical tree species precluded a rigorous experimental and quantitative analysis of all the palm seed samples received.

On arrival, fruits and seeds were stored moist in ventilated polythene bags at 16° C for up to one week before experimental treatments were begun. Where necessary, fleshy pericarps were removed by washing in tapwater. In *Orbignya cohune* the substantial, hard endocarps were removed by progressive and careful application of pressure in a large engineer's vice, with further pressure splitting the seed to permit removal of the embryonic axis.

In the control lots, seeds or embryos were set to germinate without any drying, i.e., at the relatively high moisture contents at which they arrived. Seeds or embryos were dried to low moisture content in a room at 15% relative humidity and 15° C in monolayers for periods up to four weeks. Throughout this paper, moisture contents are quoted on a percentage fresh weight basis. Measurements were made gravimetrically by weighing samples

(whole seeds were quartered) before and after drying at 103 ± 2° C for about 17 hours. Equilibrium relative humidities were measured using a Michel S-4020 dewpoint hygrometer.

Germination tests consisted of incubating seeds (and embryos in the case of *O. cohune*) on 1% (w/v) distilled water agar in either 9 cm polystyrene petri dishes or polystyrene sandwich boxes, in incubators maintained at 26° C, or fluctuating diurnally (33/19° C) with a 12-hour thermoperiod, illumination on a 12-hour photoperiod being provided by "warm-white" (Sylvania) fluorescent tubes. Incubation was continued until it was obvious that no further germination would or could occur. Palm seed germination is often quite protracted (Loomis 1958, Ellis et al. 1985) and in this work incubation periods varied from five weeks (*Washingtonia filifera*) to more than one year (*Acoelorraphe wrightii*). The tests were monitored at regular intervals, and germinated seeds (embryo extension >2 mm) were counted and removed. Whenever incipient drying out made it necessary, seeds were re-sown on fresh substrate.

In vitro techniques were also applied to embryos or seeds of *Orbignya cohune*, *Daemonorops verticillaris*, and two *Pinanga* species. After extraction from fruits, seeds were disinfected for 2 minutes in 70% ethanol, followed by 50% bleach for 20 minutes. They were then rinsed five times in sterile distilled water. Embryos were excised under aseptic conditions and individually cultured on 1 ml of MS (Murashige and Skoog 1962) medium containing 0.6% agar and 0.25% activated charcoal, in glass tubes sealed with polypropylene covers secured by rubber bands. For disinfection of excised embryos of *O. cohune*, a weaker (10–20%) solution of bleach was used. Cultures were incubated at 26° C in darkness. After 40 days, germinating seeds or embryos were transferred to fresh medium and incubated at 29° C with a 12-hour photoperiod.

For topographical tetrazolium staining of excised *Orbignya cohune* embryos, they were imbibed on paper towel wetted with distilled water for 24 hours at laboratory temperature (20–22° C), and then incubated in buffered 1% solution of 2,3,5-triphenyl tetrazolium chloride (Moore 1973) for 24 hours at 31° C in darkness. Embryos were scored as viable when they showed an overall even carmine red staining.

Results

Table 1 provides the results of the desiccation experiments. A full discussion of the results for eleven of the fourteen species examined here is presented in Dickie, Balick, and Linington (1992). Results for *Geonoma deversa*, *Jessenia bataua* and *Salacca zalacca* are added.

One species, *Acoelorraphe wrightii*, gave uncertain results. Germination of fresh seeds, as well as of seeds dried to <5% moisture content for 28 days, was erratic over a 15-month period. The viability of fresh seeds was 79% versus 17% viability for dried seeds. The greatly reduced rate of viability in the preserved seeds needs further quantification and study. However, it is felt that seed banking and cryopreservation will pose problems because of the above mentioned losses due to the drying process prior to freezing.

Discussion

Of the fourteen species of the Palmae examined so far in this investigation, the seeds of only two (*Sabal mexicana* and *Washingtonia filifera*) appear to be immediately amenable to seed bank storage or cryopreservation as means of *ex situ* conservation of genetic resources. This finding supports the commonly held view (J. Dransfield, pers. comm.) that considerable difficulties would be involved in attempts to conserve most palms *ex situ* as seeds. However, it is in contrast to data extracted from a recent literature search by Hong (1991), in which, of 21 palm species

Table 1. Palm species examined and their suitability for ex situ *cryopreservation.*

Species	Source of Sample	Tolerance to Desiccation
Acoelorraphe wrightii (Griseb. & H. Wendl.) H. Wendl. ex Becc.	Hattieville, Belize District, Belize	somewhat
Attalea crassispatha (Mart.) Burret	Haiti	no
Daemonorops verticillaris (Griff.) Mart.	Kepong, Selangor, W. Malaysia	no
Desmoncus orthacanthos Mart.	Cayo District, Belize	no
Geonoma deversa (Poit.) Kunth	Madre de Dios, Peru	no
Jessenia bataua (Mart.) Burret	Madre de Dios, Peru	no
Orbignya cohune (Mart.) Dahlgren ex Standley	Cayo District, Belize	no
Pinanga malaiana (Mart.) Scheff.	Pahang, West Malaysia	no
Pinanga aff. *polymorpha* Becc.	Pahang, West Malaysia	no
Sabal mexicana Mart. Becc.	San Antonio, Texas, USA	yes
Salacca zalacca (Gaertn.) Voss ex Vilmorin	Village market, Malaysia	no
Schippia concolor Burret	Cayo District, Belize	no
Washingtonia filifera (L. Linden) H. Wendl.	San Antonio, Texas, USA	yes
Zombia antillarum (Desc.) L. H. Bailey	Fairchild Tropical Garden, Florida, USA	no

referred to, sixteen were reported as having orthodox seeds.

Extrapolating, it could be estimated that only about 24% of palm species would bear seeds difficult to store (cf. 86% from the present study), but the work reported in the literature appears biased towards species from dry habitats (e.g., *Sabal* spp. and *Phoenix* spp.). Indeed, the results of the present study suggest that it is only those species regarded as belonging to dry habitats (*Sabal mexicana* and *Washingtonia filifera*) that bear truly orthodox seeds, easy to store at low temperatures in the air-dry state. In contrast, the remaining species examined here are characteristic of comparatively moist habitats, and it appears that the seeds of none of them would be easy to preserve at low temperatures, largely because of their inability to withstand desiccation. Of the difficult seeds, some would be truly recalcitrant, while others may belong to an intermediate category in which a certain level of desiccation is tolerated but below which loss of viability occurs. Ellis et al. (1991) have demonstrated this type of behavior in seeds of at least one palm (*Elaeis guineensis*). The work presented here has not allowed clear differentiation between recalcitrant and intermediate seed storage behavior in the species examined, although the evidence available might point to the seeds of *A. wrightii* being intermediate and those of *O. cohune* being recalcitrant (Dickie et al. 1992).

The information on seed storage behavior generated in the present study, together with that compiled by Hong (1991), represents only 31 of a total of over 2,600 palm species. As well as being very small, the sample underrepresents species from moist habitats, which probably make up the great majority of palm species. Clearly, more work is needed to establish an adequate and unbiased database of palm seed storage characteristics, which could be used to assess the utility of *ex situ* storage in individual palm species conservation programs. In the meantime, it may be possible to suggest a rule of thumb whereby those species of definitely dry habitats are highly likely to bear seeds that are amenable to dry, cold storage, whereas those from relatively moist habitats are likely to be difficult or impossible to store. Even the latter group will contain species with intermediate seed storage behavior (Ellis et al.

1991), which will allow medium-term preservation of viability in optimum environments. Also, the work of Chin et al. (1988) raises the possibility that, for species whose seeds are difficult to store, there are nevertheless *ex situ* conservation possibilities in the cryopreservation and *in vitro* culture of their excised embryos.

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