

Contrasting seed biology of two ornamental palms: Pygmy Date Palm (*Phoenix roebelenii* O'Brien) and Fishtail Palm (*Caryota urens* L.) and implications for their long-term conservation

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The Arecaceae family includes palm trees of economic importance both as a source of agricultural produce and as ornamental components in landscaping projects. Pygmy date palm (*Phoenix roebelenii*) and solitary fishtail palm (*Caryota urens*) are well known landscaping plants today. Both species have their origin in Southeast Asia and, especially *C. urens* is widespread in peninsular India and Sri Lanka. They are multipurpose species with a variety of applications and thus very heavily utilized. Knowledge of palm seed storage biology will improve their conservation prospects. In present studies, fresh seed moisture content in *P. roebelenii* was recorded to be 30% with germinability of 98%. After desiccation to 8% moisture germinability was reduced to 90% and the seeds survived cryo-exposure. Fresh seeds of *C. urens*, with initial moisture content of 34% and 95% germinability could be desiccated to lowest level of only 29% moisture content, with complete loss of germinability. Fresh as well as desiccated seeds of this species did not survive cryo-exposure. While the seeds of *C. urens* stored at room temperature lost their germinability by 110 days, seeds of *P. roebelenii* could germinate even after 9 months of storage. *P. roebelenii* is proven to exhibit orthodox seed storage behaviour while *C. urens* is found to exhibit recalcitrant seed storage behaviour. Long-term *ex situ* cryo-conservation in the form of seed gene banks would be suitable for seed propagated orthodox palm species and a few germplasm centres may be established for recalcitrant Indian palms in suitable bio-geographic regions as a complimentary *ex situ* conservation.

Keywords: *Caryota urens*, Cryo-exposure, *Ex situ* conservation, *Phoenix roebelenii*, Seed desiccation

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The Arecaceae family includes palm trees of economic importance both as a source of agricultural produce and as ornamental components in landscaping projects. The palms are harvested from wild stands and forests, cultivated in plantations and home gardens, and used in landscaping indoor and outdoor environments. Pygmy date palm, or miniature date palm tree (*Phoenix roebelenii* O'Brien), and solitary fishtail palm (*Caryota urens* L.) are well known landscaping plants used extensively at present.

The low height and graceful crown of pygmy date palm, have made it a popular accent plant in tropical landscapes as a pot plant. Because of its elegant shape, size and hardiness, with easy acclimatization to different environmental conditions, it is one of the most commonly sold dwarf palm species. It is a

popular ornamental plant in gardens in tropical and subtropical climates. The clustering habit of this hardy dwarf palm may help it to acclimatise to diverse climatic conditions and survive flooding¹. Commercially important as a container plant, grown under glass or as a house plant, it is identified as air purifying plant by NASA, as it is said to remove formaldehyde and xylene (a chemical found in plastics and solvents) from the air². Mature fruits, resembling small, thin-fleshed dates, are edible and very much liked by birds. Native to Southeast Asia, it slowly reaches 2-4 m in height and spreads as much as 3 m wide. It has an upright or curving, single trunk topped with a dense, full crown with alternate leaves, which are slightly drooping and odd pinnately compound, with leaflets arranged in a single plane.

Fishtail palm, the genus *Caryota* is the only genus of the palm family (Arecaceae), having bipinnate leaf

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anatomy. These palms have the basic shape of a feather palm, but with side branches off the main leafstalk and all the leaflets coming out of these side branches. This more complex leaf arrangement makes for some of the most dramatic and beautiful of all the palm fronds. Each leaflet of a *Caryota* palm is somewhat triangular in shape, similar to that of a fish's tail, hence the name. An extremely fast-growing species, *C. urens* (solitary fishtail palm, toddy palm, jaggery palm or kithul palm), with drooping leaves, is an attractive landscape specimen, planted mostly in the centre of a lawn or garden as a solitary palm. Native to India and Sri Lanka, *C. urens* has solitary stems. An evergreen palm, it grows up to 20 m tall and is topped by a rosette of large, gracefully curved leaves (some up to 6 metres long). The 3-metre-long inflorescences emerge at each leaf node, from top to bottom (basipetal flowering), producing pendant clusters of white, unisexual flowers. It is a monocarpic species, living for several years without flowering, but then dying once it has flowered. After about 10-15 years, flowering commences and continues for another 5 or more years. Once the last fruit on the bottom inflorescence matures, the plant dies. Solitary fishtail palm is a multipurpose tree, cultivated both for its products and as an ornamental specimen. The trunk yields starch (sago), which is eaten, especially in times of famine, by native peoples. Palms are felled to extract the central 'pith', and the sweet-tasting pulp is sundried, powdered and stored, then used for the preparation of bread by native peoples. Sap is tapped from the inflorescence, then fermented into an alcoholic drink (palm wine or toddy) or boiled down to make syrup or sugar (jaggery). The stem apex (palm heart or palm cabbage) can be chewed raw or cooked as a delicacy. Such uses have endangered the populations of this palm in its native habitat. The leaves produce strong fibres (kithul fibre) that are made into ropes, brushes and baskets. In Sri Lanka, leaves of *C. urens* are traditionally used as a 'delicacy fodder' for domesticated elephants; in areas where the trees are not tapped, they are cut down to feed elephants. The mature wood is strong, heavy and durable, suitable for flooring and making spears. As an ornamental tree, it is planted in tropical and sub-tropical gardens and also used as interior plant when it is smaller in size.

Palms are very heavily utilized by peoples. However, out of around 2700 species in the Palm Family³ detailed information on seed biology in terms

of storage and germination was available for fewer than 200 species, as reported by various researchers⁴⁻⁹. Given the restricted natural distributions, habitat loss and intensive horticultural trade in wild-collected plants, the wild populations of *P. roebelenii* and *C. urens* qualify as having 'vulnerable' status, but further studies are needed. Demand as ornamentals is mostly met through seeds and sometimes as offshoots from cultivated plants for *P. roebelenii*. However, Barrow¹⁰ suggested that collection of mature palms from the wild poses an increasing threat. Long-term *ex situ* conservation in seed banks would be suitable for palms generally propagated by seeds,¹¹ more so because other methods like *in situ* conservation faces limitations due to the fact that palms can take up to several years to germinate and exhibit variation in germination time even within the same cohort¹². *Ex-situ* conservation would ensure maintenance of genetic diversity and it is simple and economical^{13,14}. The process of natural regeneration in areas of palm occurrence is severely affected by human activity. Though *C. urens* and a few other Indian palms do find a place in some gardens, no systematic attempts have been made to popularize the particular species. Kulkarni and Mulani¹⁵ suggested setting up of a few germplasm centres for Indian palms in suitable biogeographic regions that would ensure a regular supply of their saplings along with necessary know-how for their upkeep. This can be one way of *ex situ* conservation of indigenous germplasm. Thus, a greater knowledge of palm seed storage biology will improve the conservation prospects for this highly utilized and threatened group of species¹⁶. Seed storage behaviour needs to be determined and alternative conservation technologies will need to be developed for desiccation sensitive seeds for post-harvest seed handling. In the present studies, seed biology, especially storage behaviour, has been investigated in two ornamental palms, *P. roebelenii* and *C. urens* for feasibility of their cryobanking for long-term conservation.

Methodology

Around 400 fruits of pygmy date palm (*P. roebelenii*) were collected from trees located in a garden in Gurgaon, Haryana during December. One set of about 350 fruits of solitary fishtail palm (*C. urens*) was collected from the research garden of Institute JNTBGRI, Thiruvananthapuram, Kerala during December and a second set of about 200 fruits

was harvested from palms located in the garden of IARI, New Delhi during May. Fig. 1 illustrates palm and fruits of *P. roebelenii* and *C. urens*. The pulp of the fruit was removed by rubbing them against muslin cloth and the seeds, comprising endocarp, endosperm and embryo, were extracted, washed in running tap water and kept in open in the lab (at cryo-lab NBPGR, New Delhi) for air-drying. For the purpose of the seed biology study, seed weight, length, width and thickness were recorded for ten randomly selected seeds using weighing balance and a digital Vernier Calliper. Seed moisture content was determined gravimetrically using the low constant temperature oven dry method where seeds are dried at $103\pm 2^{\circ}\text{C}$ for 17 h¹⁷. Fresh seeds were tested for viability



Fig. 1 — Palm and fruits (inset) of *P. roebelenii* (A) and *C. urens* (B).

immediately after collection. Seed germination testing was done through the Between Paper Towel (BP) method. With three replications for each experiment, 20 seeds were placed between moist filter paper and kept at 30°C . Observations were recorded at 7-day intervals. Different methods of breaking dormancy and promoting germination were tested, including dehusking the seeds, soaking the seeds in hot-water (40°C water for 15 min.), and scarification of the seeds, both mechanical (using sandpaper) and chemical (treating with 25% H_2SO_4 or 50% HNO_3 for 5 min). After standard germination tests, the seedling vigour index (Vigour Index = Germination X Seedling length) was calculated¹⁸. Seedling root length and shoot length were measured at 90 days after sowing and data recorded for 10 randomly selected samples.

Seeds were desiccated in silica gel desiccators for different time intervals and their moisture content determined. The seeds were tied in muslin cloth bags and placed over activated silica gel in a desiccator for different durations, resulting in varying moisture levels. For fast freezing, 40 seeds in two replicates for each treatment were packed in 50 mL polypropylene cryo-vials and immediately plunged in liquid nitrogen (LN). After storage for periods of up to at least 48 h, samples were thawed rapidly in a water bath at $40\pm 2^{\circ}\text{C}$ for 2 min before placing them for germination. Viability and germination testing were done for fresh, desiccated and LN exposed seeds. Each experiment was repeated thrice and the results were analyzed statistically. Standard Errors (SEs) of the arithmetic means were determined for each treatment.

Results and Discussion

Both the palms have drupaceous fruit; pygmy date palm (*P. roebelenii*) is one-seeded and solitary fishtail palm (*C. urens*) normally two-seeded. In both fruits the mesocarp is fleshy and the endocarp papery and undifferentiated. The fruits of pygmy date palm (*P. roebelenii*) are small, elliptic and violet black in colour on maturity, measuring an average of 12.45 mm long and 5.46 mm in diameter. Each fruit in our samples contained a single seed, dimensions averaging 9.96 mm long, 4.84 mm wide, 3.72 mm thick and 0.14 g fresh weight (Table 1); the weight of 100 seeds averaged 14.5 g. The seeds are elliptical, slightly flattened and with a longitudinal furrow on the ventral side (Fig. 2A). On the dorsal side, the operculum is situated near middle of the seed. The seeds are of the albuminous type, with a very hard endosperm almost completely filling its inner part (homogeneous endosperm).

Table 1 — Morphometric data of freshly harvested seeds of *P. roebelenii* and *C. urens*

Species	Seed Length (mm)	Seed Width (mm)	Seed Thickness (mm)	Single Seed Weight (gm)	Moisture Content (%)	Germination (%)	Root Length (cm) (at 90 th day)	Shoot Length (cm) (at 90 th day)	Vigour Index (at 90 th day)
<i>Phoenix roebelenii</i>	9.96 (±0.1)	4.84 (±0.1)	3.72 (±0.0)	0.14 (±0.0)	30.30 (±0.5)	98.00 (±2.0)	8.10 (±0.5)	10.96 (±0.5)	1867
<i>Caryota urens</i>	14.77 (±0.1)	11.00 (±0.1)	7.52 (±0.1)	0.99 (±0.0)	34.35 (±0.6)	95.00 (±3.0)	12.45 (±1.6)	5.95 (±0.5)	1748

Values in parentheses are ± Standard Error

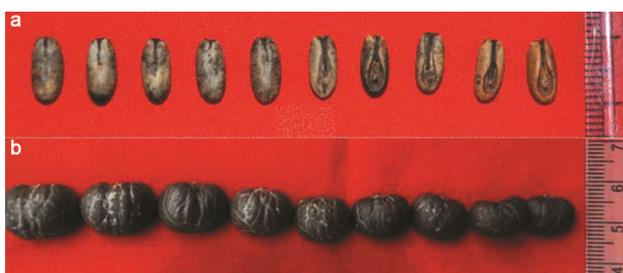


Fig. 2 — Seeds of *P. roebelenii* (A) and *C. urens* (B).

The embryo occupies a lateral and peripheral position. It is wedge-shaped (cuneiform) and its funnelled end is oriented towards the seed periphery. The embryo in palm seeds is very small in relation to seed size and seed consists of a large proportion of endosperm, a general characteristic of all palm species¹⁹.

The globose fruits of fishtail palm (*C. urens*) are dark scarlet red at maturity, measuring average 18.7 mm long, 16.7 mm wide and 15.8 mm thick. The mesocarp is fleshy, filled with irritant substances. Each fruit in our studies normally had 2 large hemispherical seeds, although sometimes only one seed per fruit was found. Seed dimensions averaged 14.7 mm long, 11.0 mm wide and 7.5 mm thick (Table 1); the weight of 100 seeds averaged 100 g. The seed surface was smooth with scattered veins (Fig. 2B), and seed was filled with a basal corky mass; ruminant endosperm. A small embryo was present on the lateral, dorsal side and it was conicus-shaped, with the pointed end oriented toward the centre.

In the case of *P. roebelenii*, the moisture content of freshly harvested seeds was 30.3%, with highest germination of 100% (average values being 98%). Germination of fresh seeds initiated on the 25th day and the last seed germinated on the 75th day of being set up for germination. In earlier studies of this species Iossi *et al.*²⁰ reported 36.5% moisture content of fresh seeds, while Castro *et al.*²¹ reported 23.6% and 22.1% moisture content for different seed lots. Pritchard *et al.*⁵ reported much lower seed moisture content (18.6%) on receipt of sample of *P. roebelenii*. Germination was reported to occur between 27 and 58 days after sowing²⁰.

Initial moisture content of *C. urens* seeds was recorded as 34.4% and their average germination was 95%. Initiation of seed germination took place between 18 to 55 days after keeping the seeds for germination. Radha²² reported moisture content of fresh *C. urens* seeds at 38.14%, with 100% germination, although they found the initiation of germination was much delayed – as late as 127 days after sowing in garden pots. Our experiments, using the BP method in lab conditions at 30°C temperature, showed germination initiation in 27 days. Wood *et al.*⁸ reported 36.4% moisture of fresh seeds of *C. urens*, with mean time to germination (MTG) at 25.3 days.

In present study, pretreatment methods using mechanical scarification (with sandpaper) and acid scarification (25% H₂SO₄ or 50% HNO₃ for 5 min) were attempted, but had no advantage in hastening germination or improving germination percentage significantly for fresh seeds of either species. According to Doughty *et al.*²³ and Odetola²⁴, mechanical scarification promotes germination of a variety of palms, especially, where the seed coat is hard. In our studies, seed soaking in hot-water (40°C hot water for 15 min) was found detrimental for *C. urens* and ineffective in improving germination for *P. roebelenii*. In the case of *C. urens*, sandpaper scarification followed by soaking in water for 24 h promoted germination, resulting in germination initiation in 15 days, with 100% germination.

Seed germination in both the species was of the remote type, in which the seedling develops at some distance away from the seed^{25,26}. In remote germination, the cotyledon petiole, after emerging out of the seed, elongates along the connective, which is termed as the cotyledonary sheath or apocole²⁷. As reported by Meerow²⁸, seed germination begins with the opening of a circular operculum, through which a bulbous structure, known as the cotyledonary petiole, is emitted. This structure is nothing but a prolongation of the cotyledon which, internally, starts to function as an absorption organ, called the haustorium. Germination in palm seeds in general is of hypogeal and cryptocotylar type²⁹. Germination of seeds of both

palm species in our research was slow and highly erratic. Seedling root length and shoot length were measured on 10 randomly selected seedlings at 90th day of being set up for germination of seeds of both the species. Seedling vigour index value was 1867 for fresh seeds of pygmy date palm and 1748 for fishtail palm (Table 1).

Seeds were desiccated for various periods and moisture content and germinability recorded at each interval. In *P. roebelenii* three desiccation periods of 18, 36 and 54 h resulted in significant reduction in seed moisture content, to 12.5, 9.4 and 8.7%, respectively (Fig. 3). A concomitant reduction in germinability was recorded with level of desiccation. Seeds desiccated to 12.5% moisture showed a decline, albeit by a marginal 5%, and further seed desiccated to 9.4% moisture and below led to a reduction of germinability to 90%.

For both the species, fresh seeds and those desiccated to various moisture levels, were fast frozen while enclosed in 50 mL cryovials by direct plunging into liquid nitrogen. Fresh seeds of *P. roebelenii*, after LN exposure, showed a rapid decline in germinability to about 73% (Fig. 3). In this species, seeds desiccated to 12.5% moisture showed a decline in germination by 5%, and further by 6% on LN exposure. Further seed desiccation to 9.4% moisture and below led to reduction of germinability to 90%, which remained unchanged after LN exposure (Fig. 4). Overall, seeds

of this species could survive desiccation and liquid nitrogen exposure with only 10% loss in germination (Fig. 3).

C. urens seeds were found to resist water loss when exposed to low moisture environment. Increasing desiccation periods (18, 36, 54, 72, 90 and 108 h) resulted in a slight reduction in seed moisture content (Fig. 3). A 4% loss in moisture resulted from 18 h of desiccation while with more time, only 1% moisture was lost despite continuous desiccation for up to 108 h. On desiccation, germination was reduced marginally (by 5-7%) with 18 h of desiccation and further germination reduced by 15% with 72 h and further it drastically dropped to 0% with 108 h of desiccation. The decline in germinability on desiccation for different periods was inconsistent (Fig. 3), indicating heterogeneity in moisture and germination potential within and between the seed lots. Previously, Wood *et al.*⁸ also reported failure of *C. urens* seed germination after desiccation to 4.8% equilibrium moisture content. For desiccation-sensitive species like *C. urens*, germination levels were similar for both the fresh and moist-stored seeds⁸. In our studies,

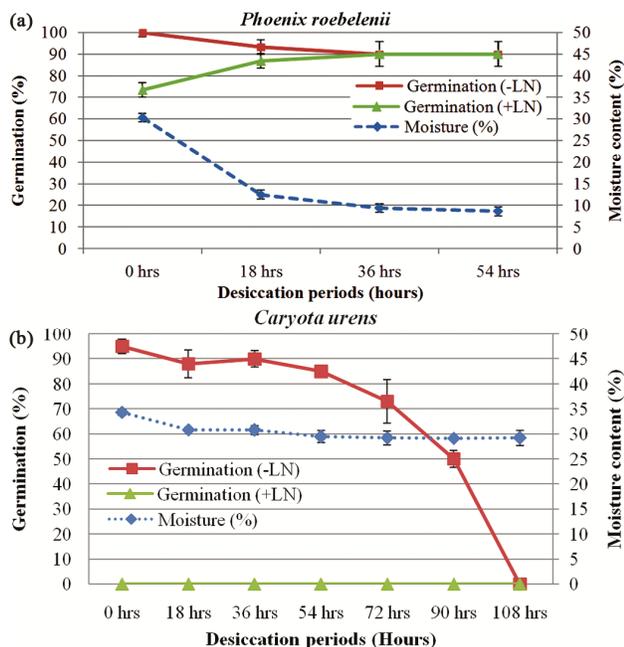


Fig. 3 — Relationship between seed moisture content and germination before and after LN exposure of *P. roebelenii* and *C. urens*

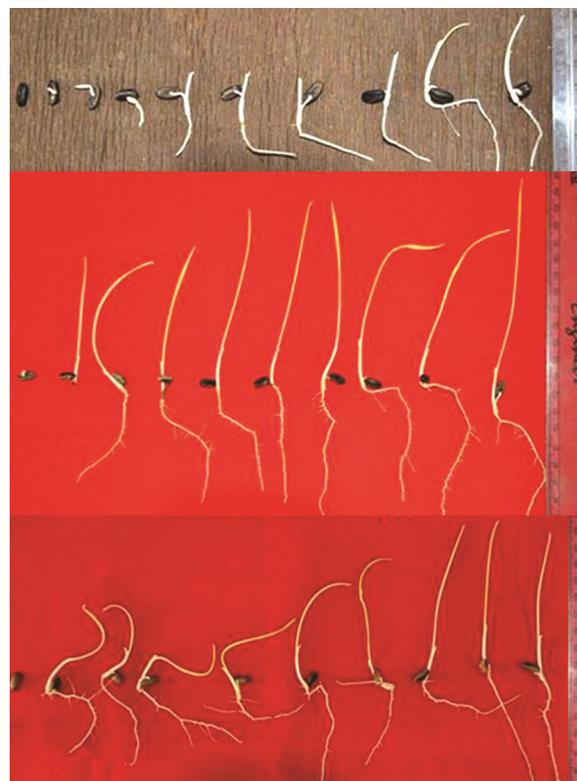


Fig. 4 — Seedlings raised from fresh *P. roebelenii* seeds after 45 days (A) and 75 days (B) of putting for germination. Seedlings raised from LN exposed seeds with MC 9.4% after 75 days (C) of putting for germination.

C. urens seeds showed desiccation sensitivity and also fresh (Fig. 5) as well as seeds desiccated up to 29% moisture content failed to survive after LN exposure.

For longevity studies seeds were kept at room temperature (RT) and germinability tested at different intervals. *P. roebelenii* seeds germinated after 9 months storage at RT without significant loss in viability. Seeds retained 95.8% germination after one and three months storage, and 94.1% after 6 months and 86.6% after 9 months when kept at RT. *C. urens* seeds, however failed to germinate at 110 days when kept at RT for longevity study. During this study seeds after 45 days storage showed 90% germination which rapidly fell to 72% germination after 90 days at RT. Previously, Radha²² had reported that fresh de-pulped seeds from fruits of *C. urens* maintained high percentage viability after desiccation for 12 weeks when kept in the open at room temperature. A decline in percentage of germination below 50% was observed only after the 24th week of storage²². According to Donselmann³⁰ and Meerow²⁸, palm seeds should be planted fresh, as their viability is lost within a relatively short period. Broschat³¹ stated that seeds of many palm species lose viability within a few weeks after harvest due to the deleterious effects of desiccation. The seeds of *C. urens* lost viability within

15 weeks while seeds of *P. roebelenii* could maintain reasonably high viability (86%) upto 9 months of storage in the open at room temperature.

Hong and Ellis³² classified seeds into ‘Orthodox, Intermediate and Recalcitrant’ based on the initial seed moisture content, tolerance to dehydration and low temperatures. According to Orozco-Segovia *et al.*¹⁹ seed longevity and storage behaviour are closely related. Orthodox seeds are those with low initial moisture content, tolerant to low temperatures (below 0°C) and have an extended viability. Recalcitrant seeds are those with high initial moisture content, no tolerance to dehydration or to temperatures and have a short viability. Intermediate seeds, on the other hand are those seeds which are tolerant to dehydration, but not to low temperatures (0°C and -20°C) and viability of these seeds can be prolonged by dehydration and ultra low temperature storage.

The classification of palm seeds on the basis of storage behaviour into orthodox, recalcitrant or intermediate is ambiguous. Seeds of many important palms are grouped under intermediate or recalcitrant category due to their short lifespan and desiccation sensitivity. Fresh seeds of *P. roebelenii* with 30.3% moisture and 98% germinability could be desiccated to 8.6% moisture with germinability reduction to 90% only and it survived liquid nitrogen exposure well with 90% viability. Thus *P. roebelenii* may be considered to exhibit as ‘orthodox’ seed storage behaviour albeit with seed longevity of less than one year. However, previous work on *P. roebelenii* by Pritchard *et al.*⁵ reported desiccation sensitivity of seeds. These authors have correlated the seed sensitivity of seeds with the growing areas of this palm species as it is found naturally growing beside the Mekong River in rainforests. Moreover, the initial quality of seed lots of this species was quite low and authors suggested detailed investigation of the relationship between moisture content and viability in this species. In contrast, our results with the seeds of *P. roebelenii*, collected from ornamental garden of humid subtropical region, due to low moisture at shedding and exhibiting marginal loss of viability with desiccation and freezing lead to categorisation of it as orthodox. Previous report on other *Phoenix* species considered seeds of *P. dactylifera* as orthodox and *P. canariensis*, *P. rupicola*, *P. sylvestris* and *P. reclinata* as probably non-orthodox⁶.

In this study, *C. urens* seeds showed very high sensitivity to desiccation and freezing. Cryo-exposed seeds could not germinate at any moisture level.



Fig. 5 — Seedlings raised from fresh *C. urens* seeds after 30 days (A), 45 days (B) and 90 days (C) of putting for germination.

Previous report on storage classification was suggested to be uncertain for *Caryota*^{8,33}. Wood *et al.*⁸ reported that *C. urens* seeds failed to germinate after desiccation. These authors suggested that the ability of the seeds to tolerate desiccation appeared to relate closely to the native habitat in which the plants grow. *C. urens* grows in wet (monsoon) climates from Sri Lanka through Southeast Asia to the Solomon Islands³⁴ and hence exhibit characteristics of desiccation intolerance. Thus the seeds of *C. urens* may be considered to exhibit 'recalcitrant' seed storage behaviour based on their high moisture at shedding and sensitivity to desiccation and freezing.

Situated in the tropical region, India houses a number of palms (represented by 20 genera and about 96 species³⁴) distributed in diverse geographic, soil and climatic areas. Collection and utilization of palms and their diverse products from the wild poses an increasing threat of over-exploitation. The process of natural regeneration in areas of palm occurrence is severely affected by human activity. Long-term *ex situ* conservation using cryobanking, the most economical and practical method, in the form of seed banks, would be suitable for seed propagated palms showing orthodox seed storage behaviour such as one of those in our study, *P. roebelenii*. It would ensure maintenance of genetic diversity with retention of original viability as has been proven by different researchers³⁵⁻³⁶. A few germplasm centres may be established for Indian palms, such as one of those in our study, *C. urens*; in suitable bio-geographic regions that would ensure a regular supply of their saplings as nursery stock for replanting along with necessary post-harvest handling of seeds as another viable alternative to *ex situ* conservation of indigenous germplasm.

Conclusion

Seeds of *P. roebelenii* showed 90% germinability before as well as after LN exposure of desiccated seeds at 8% moisture level and seeds could maintain high viability after 9 months of storage at RT. Thus, *P. roebelenii* is proven to exhibit orthodox seed storage behaviour. Considering an orthodox species, the seeds of pigmy date palm, an important ornamental palm species can be conserved for long-term as *ex situ* conservation using cryobank facility with optimal moisture between 8-10%. Seeds of *C. urens* showed very high sensitivity to desiccation and freezing and seeds could not be desiccated below 30% moisture level and thus *C. urens* is found to

exhibit recalcitrant seed storage behaviour. For long-term conservation of indigenous germplasm of these species, dedicated germplasm centres may be established besides maintaining *in situ* natural populations.

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