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Artificial pollination and fruit set in double coconut growing in India

The double-coconut palm [*Lodoicea maldivica* (J.F. Gmel.) Pers. (family: Arecaceae)] of Seychelles is one of the most interesting plant species of the world[1]. The seed of this palm resembles two coconuts fused together; hence the name 'double coconut'[2] (Figure 1 *a*). The pollination mechanism in double coconut remains unclear, with a prevalent popular belief that the species is wind-pollinated[3]. In staminate (male) flowers, nectaries are situated on the margins of the bract. Both male and female flowers emit a strong, musty, sweet smell. Only a few pistillate flowers are receptive on any palm at a given time[3]. The recent work of Blackmore *et al.*[4] has thrown light on its morphology and pollination biology.

A single plant of double coconut was raised in Acharya Jagadish Chandra Bose Indian Botanic Garden (AJCBIBG), Botanical Survey of India (BSI), Howrah (the erstwhile Indian Botanic Garden or Company's Bagan at Howrah) using seeds obtained from Seychelles in 1894 and planted in the central part of the Large Palm House. It is the only palm of double coconut that now exists in India (Figure 1 *b*); it has bloomed for the first time in the end of October 1988 and bore female flowers[5]. The inflorescence, approximately 1 m in length, persisted for about 2 years. However, there was no fruit set because of the absence of male plants. Now, the height of the plant is about 10 m and it produces one leaf per year. Presently, its crown bears 12 fully expanded green leaves (bottom three leaves are much older), one half expanded and one spear-like emerging leaf. An emerging leaf takes approximately 1.5 years for full expansion[6] (Figure 1 *c*).

In the year 2006, emergence of only two inflorescences was noticed on this tree (previous records were not kept). Thereafter, 2–4 inflorescences appeared every year. The length of inflorescences was 92.76 ± 6.81 cm (mean ± SD) and the number of female flowers in each cluster varied from 3 to 9. The total number of female flowers in each inflorescence was proportional to the length of the inflorescence. The average size (mean ± SD) of a female flower at the receptive stage was 10.83 ± 1.07 cm in length and 30.86 ± 0.99 cm across (measurement made along with perianth on 15 flowers from 7 inflorescences). The female flowers are borne singly within a pair of broad bracts and comprise of six perianth lobes, sheathing a conical ovary with sessile stigma. In the Garden, the emergence of female inflorescence is noted from the middle of March to middle of September. It emerges from inside the leaf sheath with pointed tips and slowly grows in a zigzag manner bearing several empty, incompletely sheathing bracts and the subsequent ones with complete sheathing. It takes more than a month for the full growth of female inflorescence and another 10–15 days for the female flower to attain the receptive stage (Figure 1 *d*).

Pollen grains were received in an ice box from Peradeniya Botanic Garden, Sri Lanka, where both male and female trees exist, in the beginning of October 2006. They were maintained in a deep freezer (–10°C or below)[7] until the pistillate (female) flowers reached the receptive stage. Artificial pollinations were carried out from November 2006. When the female flowers reached the receptive stage, some preserved pollen sample was taken from the deep freezer and kept at normal temperature for about 2 h for acclimatization and then dusted on the stigmatic lobes. Three rounds of pollination were carried out in the subsequent weeks as and when the stigma indicated receptivity. However, after a few months of post-pollination observation, it was realized that pollinations were not successful. Pollinations were continued during 2007–2012 without any success. In most of the cases, female flowers looked fresh for certain months; however they did not enlarge at all after pollination, and gradually dried up.

Another set of pollen grains was despatched from Nong Nooch Tropical Garden, Thailand on 1 August 2013 in a closed vial. It was stored in a deep freezer (–10°C or below)[7] till the female flowers became receptive. The viability of the pollen was also tested[8]. The pollen grains from Peradeniya always showed below 10% viability. However, the Nong Nooch pollen grains showed 69% viability. A few pollen grains were also observed under a stereo zoom microscope (Leica model-S8APO), for confirmation; the pollen grains were elliptic and monosulcate[9] (Figure 1 *e*).

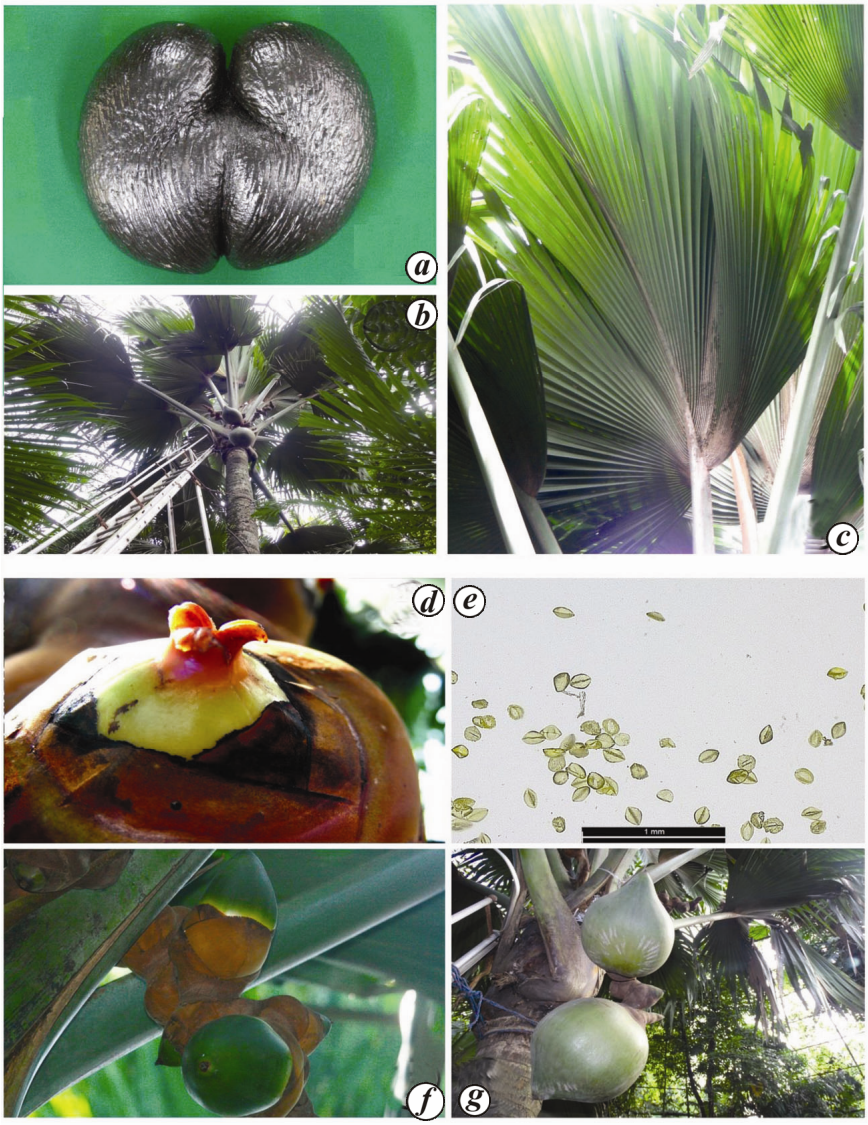

Figure 1. ***a***, A shell of double coconut preserved in AJCBIBG, Howrah. ***b***, Double-coconut Palm – habit (a ladder is placed for conducting artificial pollination.). ***c***, Photograph showing the leaf size of double coconut. ***d***, Female flower of double coconut at receptive stage. ***e***, A view of pollen under stereo zoom microscope (Leica, model-S8APO). ***f***, Young developing fruits of double coconut (3 months old). ***g***, A close-up view of 2-year-old fruits of double coconut.

On 17 August 2013, out of seven female flowers in a newly emerged inflorescence, three showed receptivity and were pollinated with the Thailand pollen sample following the same procedure described earlier. It is to be mentioned here that when new flowers at receptive stage were pollinated, the already pollinated flowers were again pollinated with some pollen in order to take maximum chance. After pollination, the whole inflorescence was pulled inside a loose white cotton cloth bag (a long tube-like cotton cloth, 1.5 m length and 60 cm width, specially stitched for this purpose) to avoid any sort of contamination. Enough care was taken to avoid touching of stigmatic heads on the cloth cover by inserting suitable vertical rods inside the cloth bag. In the meantime, the other inflorescence (containing five female flowers), which was already present on the tree and had crossed receptive stage of stigma was also pollinated by pollen received from Nong Nooch. After the pollination process, the same was also bagged as in the case of pollination done in new flowers at receptive stage. During most of the days, 2–3 h after pollination (which was usually carried out in the morning between 7 and 11 am), the palm tree experienced a lashing rain; which continued up to the evening on some days.

The pollinated flowers and others attaining receptive stage were observed twice or thrice in a day. After 3 days, another two flowers indicated receptivity and they were also pollinated in the same way as mentioned earlier. The remaining two flowers took one more week to become active. Attempts to pollinate these flowers were also made in the same manner (Table 1). It was further observed that the receptive stigma produced light-brown nectar, which was scented like a thin solution of jaggery. Once the receptivity of the stigma stopped, no more exudates were seen on the female flowers, but the scent remained for some time.

On 18 September 2013, i.e. just a month after the first round of pollination using the Thailand pollen sample, out of

Table 1. Details of artificial pollination carried out in *Lodoicea maldivica* (J.F. Gmel.) Pers. in 2013

Pollen source	Date of pollination	Number of flowers pollinated	Response
Peradeniya Botanic Garden, Sri Lanka	With the stored pollen	One inflorescence (total six flowers) produced from April to June	No change
	12 June 2013	1	(Female flowers remained green for some months and later dried-up)
	17 June 2013	2	
	21 June 2013	2	
	27 June 2013	1	
	04 July 2013	(Final attempt in all the six earlier pollinated flowers)	
Nong Nooch Tropical Garden, Thailand	With fresh pollen	One inflorescence (total seven flowers) produced from July to November	Significant changes observed in two flowers; one pollinated on 17 August 2013 and the other on 20 August 2013, a month after pollination. No changes in rest of the flowers
	17 August 2013	3	
	20 August 2013	2	
	27 August 2013	2	Measurement taken on 29 November 2013 showed the size of the bigger fruit as 31 cm in length and 58 cm in diameter and smaller fruit as 23 cm in length and 40 cm in diameter
	31 August 2013	(Final attempt in all the seven earlier pollinated flowers)	

seven flowers pollinated, two (one pollinated in the first round and the other in the second round) showed slight enlargement in size. However, the rest of the flowers remained unchanged. The observation continued in the following weeks and on 28 October 2013, it was confirmed that these two flowers enlarged significantly presumably as the result of fertilization. Further, between the two flowers enlarged, the one fertilized in the first round showed faster development compared to the one fertilized later. The measurement of the developing fruit made on 29 November 2013 showed the size of the bigger fruit as 31 cm in length and 58 cm in diameter, and the smaller fruit as 23 cm in length and 40 cm in diameter; while abortive flowers were 9 cm in length and 24 cm in diameter. Thus, the successful attempt of artificial pollination was confirmed. The fertilized fruits were vigorous in growth, emerald green and a clear light yellow uneven growth band could be seen on the parts delimiting the perianth lobes and gynoecium (Figure 1 *f*). As the gynoecium enlarged, the size of the perianth lobes appeared reduced. However, the unfertilized/abortive fruits remained dull green and the size remained almost stable throughout the observation period. A recent measurement made at the end of August 2015 has shown that the size of the bigger fruit is 52 cm in length and 112 cm in diameter, and the smaller fruit is 45 cm in length and 80 cm in diameter (Figure 1 *g*).

Thus, the present study demonstrates the feasibility of artificial pollination through international pollen exchange in this globally threatened species in India[10]. It throws a ray of hope for *ex situ* conservation in similar rare cases. Further, the study has helped enhance our current understanding on the pollination biology and natural history of this species.

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