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Determination of resource mobilization during seedling growth of palmyra palm, *Borassus flabellifer* L.

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Asian palmyra palm (*Borassus flabellifer* L.) is a multipurpose tree with year round products as food for the dependent society. Besides edible items the tree offers around 801 useful goods which are economically important. Such a plant's seedling bilogy has not been fully understood. Hence, the present study was aimed at understanding seedling developmental stages and resource mobilisation in Palmyra palm and further to determine the role of cotyledonary sheath (CS) during entire seedling growth. We investigated the developmental changes throughout growth of seedlings by providing different sets of growth conditions. Artificial seed bed made of coconut coir was compared with that of soil conditions to study germination and establishment of seedlings. Phloem loading dye was used to track the flow of nutrients from embryo to cotyledonary sheath. Seed germination in palmyra palm is hypogeal by forming ligular and tubular structures remotely in soil. Eight new organs differentiate from the seed embryo that includes haustorium, ligule, cotyledonary sheath, cataphyll, eophyll, mesocotyle (junction), primary root and mesocotyl roots during seed germination. Among these the first four are temporary organs and they disentegrate once the seedling is well established. The last four organs are responsible for developing a complete plantlet at later stages. The seed and seedling organs of Palmyra palm have four major storage reserves to support successful germination and firm establishment of seedling. Palmyra has evolved to control the solubilisation, movement and regulation of food among transient seedling organs and carry out translocation of food to the developing and differentiating organs. Seedlings also have developed physiological functions and strategies to mobilise the stored food without losing them at any point of their growth and developmental stages. Water required for seed germination permeates laterally only via cotyledonary sheath which has spongy tissues and lenticels all over. These tissues are the primary mode of water supply as the seedlings lack major root organs in the early stages of development. Flourescent microscopic and anatomical studies were carried out to observe the transport and storage of food substances required during seedling growth. Histochemical studies of seedling organs have revealed the presence of various type of nutrients such as simple sugars, carbohydrates, proteins, amino acids and lipids.

Keywords: Cataphyll, Cotyledonary sheath, Eophyll, Haustorium, Ligule, Mesocotyle junction, Seed germination

The Palmyra palm, *Borassus flabellifer* L., is a multipurpose tree, designated as the state tree (Fig. 1A) of Tamil Nadu, occupies an inevitable role in the lives of local population and other organisms[1]. It is the only species found in India out of the four listed under the genus *Borassus*[2]. All its parts serve some purpose: food from fruit and tuberous seedlings, beverage, jaggary and confectionery from the sugar sap, fibre from leaves and leaf sheaths for brushes, brooms, furnitures, weaving and plaiting, trunk wood for construction and fuel and numerous minor products valuable to the human society and also in documentation in ancient period[2-6]. Recently, Pammi *et al.*[7] has reported the therapeutic and probiotic attributes of traditional Toddy Palm Nectar (TPN). Seed germination of palms in general is hypogeal by forming ligular and tubular structures remotely in soil[8]. Dassanayake & Sivakadachchan[9] have reported the structure of embryo, seedling and mode of germination in Palmyra palm. During the palm seed germination process, cotyledonary sheath plays a vital role in transport of nutrients and water to the developing cataphyll. Morphophysiological changes[10] that occurs during the development of cataphyll in the case of *Corypha umbraculifera* seedling has been documented. Presence of polyembryony and production of twin seedlings as a rare phenomenon in palmyra palm has also been reported[11]. Occurrence of two or more branches as unusual formations though rare in *B. flabellifer* is also reported[12].

Endosperm is the main reserve source of plant polysaccharides. It serves as a food reserve for the germinating seeds and prevents complete drying up of

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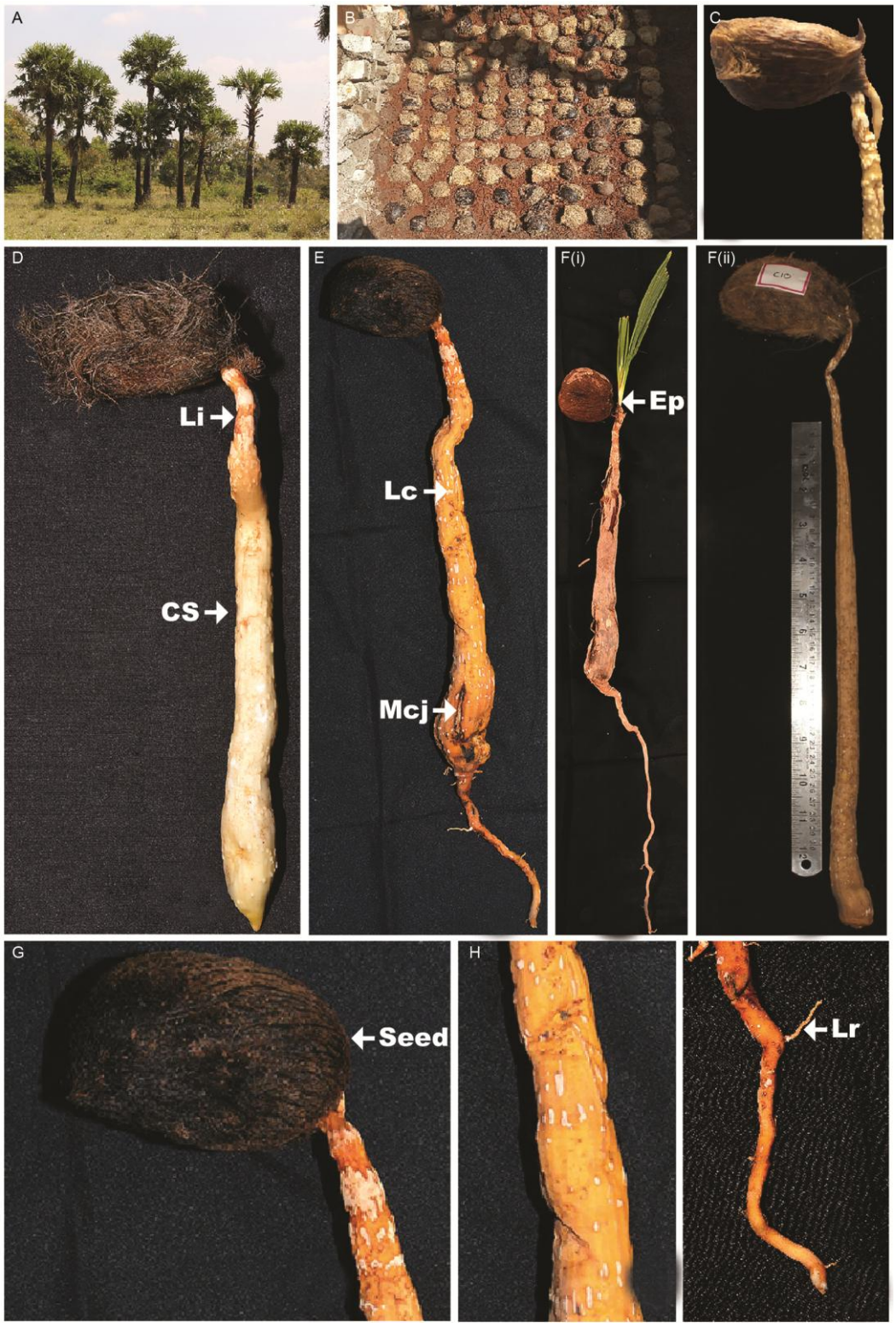

Fig. 1 — Palmyra palm seed germination. (A) Palmyra palm (*Borassus flabellifer*); (B) Seed bed with soil; (C) Twin seedlings due to Polyembryony; (D) 25 days old seedling with Cotyledonary sheath (Cs) and Ligule (Li); (E) 100 days old seedling from soil with Lenticel (Lc) and Mesocotyl junction (Mj) and normal root; (Fi) 180 days old seedling with emerged Eophyll (Ep); (Fii) 75 Germinated seedling without developing root in coconut coir; (G) Seedling with terminal portion of cotyledonary sheath; (H) Middle part of cotyledonary sheath with a lot of lenticels; and (I) Primary and Lateral root (Lr) develop from mesocotyl junction.

seeds by retaining water and thereby preventing protein denaturation including those of the enzymes involved in seed germination. The important storage of carbohydrates in palm seeds are the complex polysaccharides like mannan, galactomannans and glucomannans or glucogalactomannans found mainly as cell wall components of the endosperm[13,14]. The resting seeds of most palm species contain little or no starch but are rich in polysaccharide reserves referred to as cell wall storage polysaccharides (CWSP) which are of wide spread occurrence in seeds. These include groups such as mannans, xyloglucans and galactans. The presence of galactomannans and mannans in the solid endosperm has also been reported[10,15-18]. The reserve polysaccharides occur mainly as cell wall components of endosperm[10,13,14,19].

According to Mukherjee *et al.*[20], galactomannan of *B. flabellifer* is composed of D-galactose and D-mannose in the ratio of 1:2.4. Mannans are the main reserve materials in the seed endosperm of Arecaceae family[21]. Main storage reserves of palm seeds are lipids and insoluble polysaccharides[22,23]. Many palm seeds contain very large amount of lipids. During the early stages of germination, carbohydrates are metabolized more rapidly than the lipids, but during seedling development, the cotyledonary hasutorium converts triglycerides to carbohydrates[24]. In other palms, the endosperm itself digests the stored reserves, which are subsequently absorbed by the haustorium[25].

Germination pattern and seedling morphology of palm seeds, especially those of ligular remotive type are unique and interesting. The ensuing reserve mobilization warrant profound attention because of the convoluted sequence of mobilization of food reserve from the endosperm to seedling organs involving different morphological components of seedlings and the longer time span, compared to other seeds. Viji[26] has reported germination pattern, storage behaviour, desiccation and reserve mobilization of palmyra palm seeds. However, a comprehensive report on the transport mechanisms and seedling biology of palmyra palm is not available until now. In this study, we investigated the developmental stages of Palmyra palm to understand the morphology of seedling, seed germination, and the movement of water from surrounding soil to the growing radicle region as there is no proper root system during the initial stages.

Materials and Methods

Palmyra palm seeds were collected from Madurai, Coutrallam and Agarakattu of Tamil Nadu region. About 100 seeds were used for germination in soil as well as seed bed made of coconut coir to study the developmental stages of Palmyra palm seedlings. During the seed germination process, eight new organs differentiated from the seed embryo that includes hasutorium, ligule, cotyledonary sheath, cataphyll, eophyll, mesocotyle (junction), primary root and mesocotyl roots. The study samples like Upper part, middle part and terminal part of the cotyledonary sheath, endosperm and haustorium from germinated seedlings were collected and fixed in fixative formalin-aceto-alcohol (FAA)[27] for anatomical investigations. Fresh materials obtained from department nursery were also used for anatomical and biochemical studies.

Seed bed preparation

A seed bed was prepared (5×3×3 feet) in order to study the seed germination pattern in Palmyra palm and provided with different substrate conditions (Table 1). The seedbed was prepared and 100 Palmyra palm seeds were sown. Starting from 25 days to 75 days with 10 days of interval few seedlings were taken out and morphological changes in all parts of the seedling such as endosperm, haustorium, cotyledonary sheath, pneumathods and the development of cataphyll and eophyll were observed for changes and recorded. The time duration for emergence of first leaf above the ground was also observed. Since we wanted to understand nutrient mobilization during the seed germination process,we ntoed down all the morphological differentiation of palm seedlings.

The half of the seed's mesocarp from the collected palm seeds was removed using sharp knife, fresh weight was noted, and the seeds were dried for 10 days in sunlight in order to remove moisture content. After drying the seeds for ten days, dry weight was determined and tabulated. Coconut fibre was procured in bulk and was boiled using large vessels to remove

Table 1 — Seed bed conditions provided

Nature of Palm seed	Seed bed provided	No of palm seeds
With mesocarp	Wet (Control - sand)	10
Without mesocarp	Dry (Control - sand)	10
With mesocarp	Wet (Coconut fibre)	10
Without mesocarp	Wet (Coconut fibre)	10
With mesocarp	Dry (Coconut fibre)	10
Without mesocarp	Dry (Coconut fibre)	10

tannin contents from it to ensure no exchange of nutrients. Then using seed germination boxes, seeds were grown under the specific set of conditions as mentioned above.

Experimental setup for Seed germination

Palmyra palm seeds with mesocarp were taken and provided with necessary suitable conditions in seed germination boxes, once in every four days water was sprinkled with the amount of 400 mL. After 70 days of seed germination process, seedlings were collected and weight was measured for cotyledonary sheath and along with seed.

Seed germination percentage and Biomass was calculated using following formulae.

$$\text{Survival percentage} = \text{Total no of germinated seeds} \div \text{Total no of seeds} \times 100$$

$$\text{Biomass} = \text{weight of the cotyledonary sheath} \div \text{weight of dry seed} \times 100$$

Seedling clearing protocol

From seedbed two months old seedling sample was taken and the length of cotyledonary sheath was measured prior to clearing 3-5 cm. Seedlings was carefully removed from the endosperm and endocarp but along with intact haustorium and cotyledonary sheath. The selected seedlings were stored in 95% ethanol[28] for one day with regular changes of ethanol 95% for every 8 h. The seedlings were stained with 1% basic fuchsin in 95% ethanol for 24 h, washed in distilled water, and immersed in 5% sodium hydroxide solution in an oven at 60°C for 3 days. Twelve washes were performed with distilled water with changes every 30 min[29]. The material was dehydrated in ethanol series and stored in 70% ethanol.

Preparation of histochemical stains and reagents

Specific stains and reagents as listed in Table 2[30-36] were prepared fresh for staining hand sections as per the standard methods described by the authors cited. The histochemical studies were carried out using light and fluorescent microscopes (Table 2).

Microscopy and Photography

All selected thin sections were stained with above mentioned dyes and reagents and observed using Nikon *80 i* Eclipse microscope fit with light, dark field, phase contrast and fluorescence microscopic facilities. Micro photography was done using a camera attached with the microscope facilitated with image – one software NIS-Elements BR. Selected good pictures were grouped as plates.

Biochemical analysis

Haustorium tissue samples were used to extract carbohydrates and tested with anthrone's reagent in order to check the presence of carbohydrates. The haustorium has abundant of starch grain bodies. In order to confirm the presence of carbohydrates qualitatively, anthrone reaction was carried[37]. About 0.5-1 mL of the test solution was added to 2 mL of anthrone reagent and mixed thoroughly. Reactions were observed for the colour changes of bluish green by keeping them in boiling water. Here, the furfural compounds reacts with anthrone to give bluish green coloured complex.

Tracking resource mobilization in palmyra palm seedling

The Palmyra palm seedling which is about 2 inches (5 cm) in size was selected and safranin dye/phloem loading dye was injected using 1 mL syringe at the ligule and seed junction (1 cm below) of the palmyra palm seedling. Repeated injection was done for about five days at one day interval. Such injected seedlings were taken for vein clearing processes to study the direction of water movement and reserve food translocation[38].

Results and Discussion

Developmental Biology of Palmyra palm seedlings

Palms have a unique characteristic feature in seed germination process. Tomlinson[39] recognized three main types of germination in palms, according to the degree of extension of different parts of cotyledon and presence of an additional structure, the ligule *i.e*, remote tubular, remote ligular and adjacent ligular. *Borassus flabellifer* belongs to the remote tubular type of germination. In this type, embryo is straight and cotyledon extends so that the plumular portion of a seedling is carried away from seed. The pattern of seed germination described by Dassanayake & Sivakadachchan[9] has some clarity. With new terminologies[40], we have made an attempt for further clarification based on our observations. Seed germination in Palmyra palm is hypogeal, meaning

Table 2 — List of Stains and Reagents used

Stain	Stained region/cells
Safranin[30]	Cell nuclei
Iodine Potassium Iodide[31]	Starch grains
TB'O (Toluidine blue O method)[32]	Lignin. cell wall
Coomassie brilliant blue G250 or CBB method[33]	Proteins
Basic fuchsin[34]	Cytoplasm and Nuclei
Acid fuchsin[35]	Protein
Xylidine ponceau[36]	Lipid reserves, Cytoplasm

much of the germination process and seedling organs occur below the soil (Fig. 1 B, D-G)[39]. As the seed germinates remotely by forming a ligular and tubular structure close to the seed (Fig. 1D), the root and shoot primordium are carried away from the seed and buried in soil, this type of germination has been envisaged as remote ligular/tubular as described by Moore Jr & Uhl[41]. Remote-ligular/tubular germination has been considered as the most advanced type of germination in the tribe Corypheae to which the Palmyra palm belongs.

Palmyra being a monocot the seed, embryo and related seedling organs have been described with many terminologies related to dicotyledons[42]. In this study, it is redefined with justifiable terminologies applicable to monocotyledons in the recent times. Plumular-radicular axis = Shoot – root axis; Primary root (Pr); secondary roots = adventitious/mesocotyl roots, pneumatophore roots[43]; (Fig. 1 D-F & I) hyperphyll = haustorium; ligule (Li) = cotyledonay petiole; cataphyll/scale leaf; Cotyledonary sheath (Cs) (Fig. 1 D-F); (tubular structure which has leaf and stem characteristics, encloses the cataphyll, shoot – root aixs, eophyll); Eophyll (Fig. 1 F(i)) = the first leaf with a blade and which can photosynthesize; rolled inwardly and appears like a cylindrical stalk in the middle of cataphyll; pneumathodes = lenticels. During seed germination process, eight new organs are differentiated from a matured seed embryo that includes hasutorium, ligule, cotyledonary sheath, cataphyll (Cp), eophyll, mesocotyle (junction) (Fig. 1E), primary root and mesocotyl roots or adventitious roots.

Seed and seedling organs of Palmyra palm have four major storage reserves to support firm establishment of the seedling at any kind of terrestrial habitats. It has evolved to control the solubilisation, movement and regulation of food at these transient organs and carry out translocation of food to the developing organs. It has developed certain physiological functional strategies to mobilise the stored food without losing them at any point of growth and developmental stages of a plantlet.

The first major storage organ of the palm seed is the solid endosperm (Fig. 2E) which also harbours the embryo and cotyledon within at one of the poles and always closer to the micropylar region. From endosperm the food moves to the Second transient storage reserve - the haustorium (Fig. 2 E & F), that grows inside a cavity of the endosperm. Later the transformed food moves to developing cotyledonary sheath (Fig. 1. D-F & Fig. 2 A & B) — third storage site which is also the third semi transient storage organ. The fourth solid food storing seedling organ is the cataphyll/scale leaf/tuber (Fig. 2 B & D) which draws all food from the former three temporary storage reserves and distributes the same to developing shoot, root meristems and leaves in a

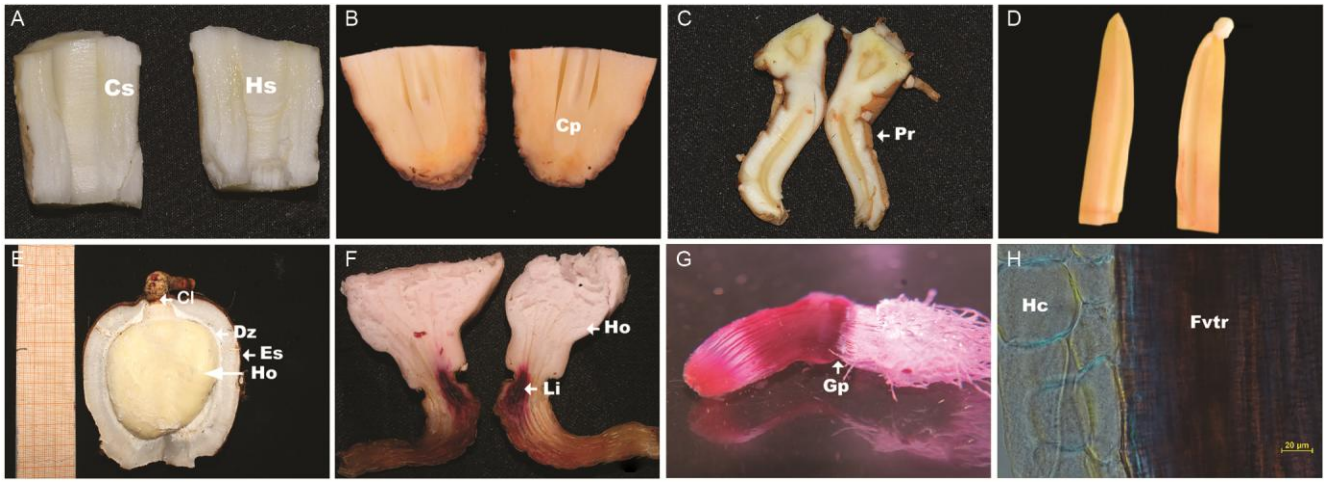

Fig. 2 — Differentiation of seedling organs and demonsation of flow of nutrients. (A) Longitudinal sectional view of cotyledonary sheath (Cs) with a Hollow space (Hs); (B) Longitudinal view of cotyledonary sheath along with emerging cataphyll (Cp); (C) Longitudinal view of primary root (Pr) from a seedling; (D) L.S. of Cataphyll consists of tuft of hairs at the top; (E) Safranin injected seedling along with haustorium (Ho), Digestion zone (Dz), Collar (Cl) and endosperm (Es); (F) Longitudinal section of cotyledonary sheath along with haustorium injected with safranin; (G) Macerated young seedling of Germpore (Gp) stage shows the flow of food from haustorium to cotyledondary sheath; and (H) Haustorial cells (Hc) connections close to Fibro-vascular traces (Fvtr).

gradual manner. The life of cataphyll is for about one to four years or till the establishment of about four – six leaves. On exposure to sunlight cataphyll too becomes photosynthetically active by developing green colour.

During initial seed germination proximal region of the cotyledon enlarges and elongates into the endosperm, the endosperm (Es) is hydrolysed by enzymes and transfers the food to a growing hyperphyll - the haustorium (Ho) (Fig. 2 E & F). This organ gradually enlarges into the central region of the endosperm and finally fills up all the space previously occupied by the endosperm to form a spongy aerenchymatous structure traversed by vascular bundles. This haustorium functions as a transient food reserve for the developing cotyledonary sheath and cataphyll. Meanwhile the region between the proximal part and the apical shoot primordium elongates forming the cotyledonary stalk. This one carries the hidden shoot primordium and root apex at its tip and forcing it way through the germ pore, grows downwards into the soil. Initially the germ tube comes out first as and when the seed finds some moisture with the cotyledonary petiole which extends its growth as cotyledonary sheath (after 20-25 days of germination). The cotyledonary stalk develops into a solid cylindrical structure, often marked externally by pneumathodes[9] and bearing at its tip the primary root, which tapers to a point. The distal part between shoot meristem and cotyledonary stalk is void. This is the sheathing portion, which encloses the shoot primordium at its distal end (Fig. 1D).

In the distal region, the cotyledonary sheath encloses major differentiating organs such as shoot primordium, root primordium at the mesocotyl junction. In aged seedlings, (45 days of seed germination) the hallow structure elongates further with the growth of cotyledonary sheath around it, and scale leaf/cataphyll (functions like coleoptile) is seen, lying within the hollow, and with a tuft of hairs at its tip (Fig. 2D). These hairs probably help to protect and lubricate the tip of the scale leaf during its growth through the empty space. The external surface of the cotyledonary sheath is continuous distally with that of a radical like structure. Later, the radicle develops into a primary root (Fig. 2C), followed by thin lateral roots growing out from the thick collar like basal region which we term it as mesocotyl junction (Fig. 1E).

The cotyledonary sheath is positively geotropic (Fig. 1D) whereas the cataphyll (cotyledon like) is strongly negatively geotropic (Fig. 2 B & D)[43] which is a very unique form of growth and development and both these become firm and hard and the latter grows to a thickness of about 4 cm at the base. However, the cotyledonary sheath alone is about 1-3 cm from top to bottom at different phases of growth. The fully grown cataphyll enlarges to a size of about 30-50 cm long and about 3-4 cm in breadth at its broadest part. It has no lamina but consists of only a thick sheath with a deep groove down its ventral side, and it tapers to a point at its apex on the dorsal side. The cataphyll is covered by a thin papery epidermis which can be easily peeled, (65 days of seed germination) contains typical leafy epidermal cells and scanty stomata. These stomata are non functional. The cataphyll is hollow, with a narrow channel about 4 mm broad down its centre which is occupied by the first photosynthetic foliage leaf (eophyll) and, lowers down, by the shoot primordium, and it is thicker on the dorsal side and laterally than on the ventral side.

The anatomy of cataphyll, beneath the epidermis there are three layers of thick- walled parenchyma cells which are devoid of starch grains and beneath these cell layers is a ring of longitudinally arranged bundles of sclerenchyma fibres which are clearly visible externally as fine brown parallel lines (Fig. 3H). The central region is occupied by large thin-walled parenchyma cells are fairly and loosely packed with intercellular spaces and these cells are densely packed with starch grains (Fig. 3 C-F). A number of vascular bundles accompanied by sclerenchyma fibres traverse this region longitudinally (Fig. 3H). This scale leaf is the edible part of a seedling, known as "Kizhangu" in Tamil (kelingoo/ tuber/cataphyll/scale leaf) locally in Srilanka and India[9].

After the differentiation of cataphyll, it is the growth of eophyll which develops inside the cataphyll, and has a tuft of hairs at its apex (Fig. 2D). Eophyll grow up and emerges through the tip of cataphyll. As eophyll enlarges, the cataphyll springs and becomes ruptured along the groove down its ventral side, where the tissues become withered and turns brown.

The first foliage or eophyl emerges above the ground in a seedling after 6-12 months of growth. The eophyll has thick brown hairs at the tip which help

them to pierce through the cataphyll first, then the cotyledonary sheath and later comes above the soil. This leaf has an oblanceolate plicate lamina[44] and is about 8-25 cm broad with a truncate apex split into 5-7 teeth and with five-seven clearly marked veins. Later leaves tend to be narrowly oblanceolate to linear, longer and larger. The leaf apex split into two three broad fan like structures but united at the base. From the second leaf onwards the leaf petiole develops spiny serrate margins on either sides of petiole[40,44].

Rarely a single seed has produced two seedlings from the germpore and both with normal cotyledonary sheath which was an unusual observation in Palmyra palm. Such seeds may have two embryos or fused carpels and hence, the resulting seedlings are abnormal. Polyembryony and multiple seedlings (Fig. 1C) have been reported previously in Palmyra palms[11].

Morphological Changes in Cotyledonary sheath and its functions

As the seed germinates cotyledonary sheath (CS) is the major seedling organ that under goes dramatic changes in its outer morphology and anatomy as a result of dynamic functions of seedling. As and when the cotyledonary sheath is differentiated the outermost layer develops a lot of lenticels (pneumathodes) (Fig. 1 D, E & H) for the exchange of air and absorption of water[45] from the surroundings. As the seedling organ is lacking a proper root at this point of time, the cotyledonay sheath functions almost like a normal root till the primary and lateral (mesocotyl roots) roots are developed. From 25 to 45 days of germination, the cotyledonary sheath was super active for absorption of water and nutrients from the surrounding soil. Internally, the CS is vibrant in many functions that includes transport of water to endosperm and haustorium in upward direction, distribution of nutrients from haustorium to growing seedling organs like mesocotyl junction, cataphyll, eophyll[46] and later leaflets in a downward direction.

After 45 days of growth, differentiation of shoot initiates by forming a cataphyll primodium from mesocotyl junction at the bottom of the seedling or cotyledonary sheath (CS) which grows against gravity and shows phototrophic nature. The cataphyll enlarges inside a tubular empty space of the CS. At the same time from 45 to 55 days, there is differentiation of primary root primordium below the mesocotyl junction which in fact the CS extends itself and forms a tubular hallow space downwards for the primary root to grow and fill the space. However, the root primordium may be very minute at this point of time but later, the root elongates and fills the hallow space after 65 to 100 days. From 100 to 140 days the mesocotyl junction differentiates many more additional roots and they all function like pneumatophores, structurally and functionally similar to that of certain other plants and palms.

From 3-6 months of seedling growth, the CS shows drastic changes both internally and externally[10]. In the outer surface, lenticels enlarge and sometimes break lengthwise to give more space for cataphyll to expand. From six months to one year, the CS starts degenerating because by this time endosperm and haustorium would exhaust their food supply and they would have become resourceless. From then onwards, the CS loses its nature of supplying water upwards to the above said tissue storage organs. Once the downward movement of nutrients stops the major function of CS will also ends and try to out fill its resources to the cataphyll and begin degeneration of its tissues. Thus, over a period of 12-15 months, the CS loses all its resources including the fibrous sheaths and appears papery or sometimes just the fibres alone are found and even the outer papery remnant of CS disappears. Hence, the CS acts like a major bridging organ of linking the haustorium and cataphyll till the later becomes independent of protecting itself from soil and soil born organisms. Such a developmental dynamism has also been observed and reported in many other palms[47].

Effect of providing different set of growth conditions on the Palmyra palm seed germination & Biomass

To check the mode of water uptake and eventually quantify the biomass generated out of 75 days old seedlings of Palmyra palm was tested by sowing seeds in wet sand (control) and coconut coir beds separately[48]. Seeds were sown during end of January and harvested in the first week of April that is nearly about 75 days old. Seeds were sown almost in the half season because the actual season for germination is during September. The results obtained are summarised in Table 3. The wet conditions were watered sufficiently and dry conditions received only 50% of water on daily basis. Only in control wet condition 50% of the seeds germinated and

Table 3 — Biomass produced by 75 days old seedlings of Palmyra Palm

Treatment	Fresh wt. (g) (A) Average±SE	Dry wt. (g) (B) Average±SE	Difference in moisture content (C) Average ± SE	% Germination out of 10 seeds	Entire seedling wt. (g) after (D) Average ± SE	Cotyledonary sheath wt. (g) (E) Average ± SE	Biomass (g) (F) Average ± SE
Sand bed - Control							
Seed with mesocarp	222.4± 13.14	182.7± 3.75	42.7± 10.47	0	-	-	-
Seed without mesocarp	348.6± 8.78	214.4± 4.71	134.2± 8.59	50	205.2± 4.30	37.15± 4.44	17.23± 1.94
Coconut coir bed – Wet condition							
Seed with mesocarp	251.5± 29.16	192.9± 8.78	586± 22.27	0			
Seed without mesocarp	894± 20.50	191.33± 18.35	106.66± 4.85	30	162.9± 13	3.91± 1.70	2.22± 1.12
Coconut coir bed – Dry condition							
Seed with mesocarp	280.5± 14.82	185± 9.52	95.5± 7.28	0			
Seed without mesocarp	297.66± 19.61	179.67± 31.89	109± 8.96	30	158.7± 10.39	0.80± 0.288	0.47± 0.12

produced about 17.23 g of fresh weight biomass[49] after 75 days. The biomass includes CS, cataphyll mesocotyl and eophyll. In all the cases, the root primordium did not differentiate. The terminal region of CS remained blunt and lacked primary root primordium [Fig. 1F(ii)] This finding is quite interesting because all the water required for seed germination have permeated only via CS which had spongy tissues and lenticels all over. Hence, these tissues were the only mode of water supply as the seedling lacked true root organs. The seed bed created by keeping coconut coir appeared to be blocking the normal seed germination though it did not affect the movement of water to the growing seedlings as reported earlier[12]. Earlier, seeds were simply laid over concrete floor and the seeds did germinate with an abnormal seedling without root and cataphyll[12].

To track the flow of nutrients from haustorium to seedlings, injection of a phloem loading dye solution was subjected at the ligule region after 55 days of germination (Fig. 2 E-G). The dye could stain vascular strands and mobility of nutrients was towards growing basal region of CS that is downward movement. Longitudinal section of the injected portion along the CS was made to observe the details under the stereomicroscope. The flow of nutrients was more towards the growing tip region (Fig. 2G). It is suggested that future experiments shall continue to repeat this kind of tests and confirm the flow of nutrients till mesocotyl junction and cataphyll.

Anatomical and Histochemical localization of various types of nutrients

As per the procedures described by Mazzottini-dos-Santos *et al.*[28] the cotyledonary sheath along with haustorium were macerated together. The connection between haustorium and cotyledonary sheath was observed under stereo microscope for anatomical features. The macerated fibres appeared pinkish in colour because of basic fuchsine. These bundles of fibres are instrumental for making a very strong link and transport route for the mobilisation of reserve food from endosperm to haustorium and later into developing seedlings.

The endosperm of palmyra palm is made up of very thick walled cells which ultimately store non starch cell wall polysaccharides such as polygalacton, galactomannan and xyloglucans[50]. As the seed starts germination primarily the endosperm cells from the centre region and its periphery undergo continuous hydrolysis reactions due to hydrolytic enzymes. This leads to crude digestion of endosperm (Es) cells and forming a slimy nutrient solution at the digestion zone (Dz) (Fig. 3 A & B). Simultaneously the central lacuna of the endosperm is being filled with the growing haustorium (Ho) which is part of the embryo situated at the micropylar pole. The haustorium forms several vascular bundles (Vb) (Fig. 3G) inside and they are connected with growing cotyledonary sheath outside the seed. The haustorium absorbs digested nutrients from the endosperm sidewise and there are no specialised cellular connections between these to

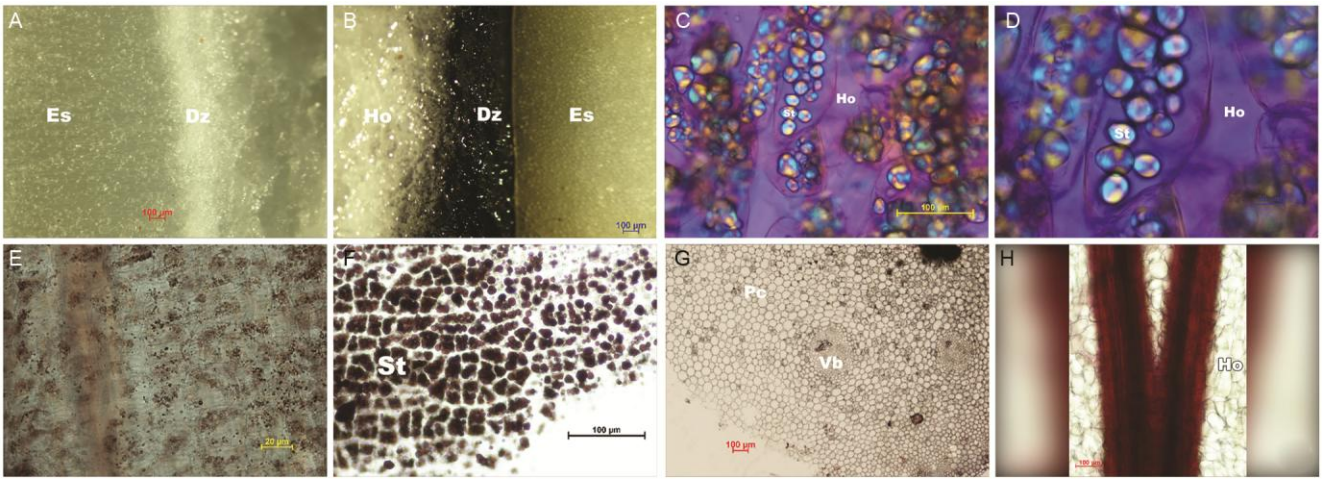

Fig. 3 — Histochemical localizaeion of nutrients. (A) Endosperm (Es) with a digestion zone (Dz); (B) Haustorium (Ho) stained with I_2Ki indicates the presence of Starch; (C & D) Pack of Starch grains in polarised view with first order red plate (St) in Haustorium (Ho); (E) Terminal region of Cs stained with I_2Ki (40X); (F). Haustorial epidermal peeling stained with I_2Ki shows starch grains (St); (G) Cross section of cotyledonary sheath (ternminal region) showing Parenchyama cells (Pc) and Vascular bundles (Vb) with less of starch grains; and (H) Macerated single fibre bundle (Fb) stained with basic fuchsin

transport the nutrients (Fig. 3 A & B)[28]. However, close observation at the vascular bundle and haustorium cells reveals the route by which transport of nutrients move into haustorium. The haustorial marginal wall cells have plenty of plasmadesmata by which the nutrients flow inside and continue to do so till the endosperms is exhausted (Fig. 3B). The digestion zone is found to have simple sugars, amino acids, proteins and very minute level of lipids. Histochemical tests confirmed the presence of simple sugars and proteins at this digestion zone as well as inside the haustorial tissues (Fig. 3 B-F). Simple sugars were abundant in the digested zone and macerated fibre walls and haustorial tissues (Fig. 3 B, E & F). Similar observations were described in *Acrocomia aculeata* by Mazzottini-dos-Santos *et al.*[28]. Qualitative tests for proteins and sugars also have confirmed the abundant presence of the same in hausotrium and digested zone tissues.

The reserve food from endosperm moves to haustorium where it is temporarily stored and distributed to the growing seedling organs via CS. As the seedling develops, the mobilization of polysaccharides intensifies and the digestion zone gradually increases. As the polysaccharides, temporarily stored in the haustorium, are mobilized, there is an increase in mobilization of polysaccharides from the endosperm, including those of the remnant cell walls, which are mobilized more slowly, forming thick layers of collapsed cells. In the next phase, sugars were identified in the endosperm, in the epidermis of the haustorium, and in the vascular bundles. Throughout the growth of a seedling, there are alternating phases between the sugars of the endosperm being mobilized more intensely to the haustorium, and from there to growing seedling, and this dynamics continue throughout the seedling developmental process. The macerated fibres of CS showed the presence of proteins stained with xylidine ponceau.

The connection between haustorium and cotyledonary sheath is formed by developing vascular and fibrovascular bundles from embryonic region[51]. To trace the connections entire portion of a cotyledonary (35 days old) sheath along with one fourth portion of haustorium were subjected to maceration and vein clearing process. The results exhibited are quite interesting where the number of fibrovascular bundles found in the CS is same to that of vascular bundles present in the embryo and haustorium. The macerated product was stained with basic fuchsin and that showed the clear distinction of vascular traces. The same number of vascular traces leads down till the mesocotyl junction and connect three major tissue organ regions such as haustorium, cotyledonary sheath and mesocotyl junction. The vascular bundles of the CS are formed as fibro vascular bundle as it required a lot of strength to hold the growing seedling. The fibrovascular bundles are formed by increasing many layers of sclerenchyma

fibres around the vascular bundles. Whereas the vascular traces of haustorium does not have any trace of sclerenchyma fibres and they appear very thin. The haustorial vascular bundles possess more of phloem[52] thin walled cells and a few xylem cells, metaxylem has not differentiated. These are primarily evident because to the haustorium and endosperm water supply is required very minimally and thus xylem is lesser in VB. However, phloem cells are many as they are required to transport the nutrients down the seedling via CS hence, they are abundant in haustorium. All these findings are reported for the first time and hence, new to the known science of palmyra palm.

In contrast to the haustorial vascular bundles and the CS vascular bundles are highly differentiated as fibrovascular bundles (FVB)[53]. The FVB of CS at early stage (25 days of germination) they are not lignified or sclerified, but sclerenchyma cells have had well differentiated during this time. Lignification and thickening of the same cells happen after 35 days of germination. The total number of FVB is similar to the number of vascular strands present in haustorium or at the early stage of embryo itself. The cross sectional view of CS after 25 days represent enormous amount of simple sugars and proteins in the parenchyma cells (Pc). If these nutrients are not utilised quickly they are converted as storage sugars like starch in the CS itself. These storage sugars are temporarily stored here for some time and later utilised by the growing cataphyll and its associated seedling organs[50,54]. The differentiation of xylem in the CS is significant because this xylem will transport water to the haustorium and endosperm for rapid hydrolysis.

The haustorium though functions like a transient reserve food organ eventually stores simple sugars, proteins, amino acids and traces of lipids absorbed from the adjacent endosperm. These nutrients move downwards to the CS till the mesocotyl junction and later in to the cataphyll. If these nutrients are not transported quickly the hasutorium converts them into storage food reserve and hence starch (Fig. 3 C-F) formation takes place and stored in every cell. The epidermal peelings of haustorium depicts the occurrence of simple sugars as well as storage sugars like starch in the surface layer itself and it is the same in all the internal tissues of haustorium. The hasutorium and CS have very peculiar way of storing and transporting nutrients from the endosperm. They keep switching the nature of storing and transporting nutrients as per the demand of the cataphyll, eophyll and later foliages. Finally the entire endosperm gets exhausted its storage reserve and moves them to haustorium. After 180 days of germination the endosperm cover - the pericarp becomes like a papery shell. The haustorium too gets transformed as a very thin layer of dead membrane once all the energy moves downward to the CS. To our surprise the CS also transfer all the food reserve to cataphyll and juvenile leaves and shoot primordium. At one point of time the cataphylls also translocate the food reserve and store them in tender leaf primoridum, shoot primordium and leaf petiole. All these portions are well protected in the soil so that life of the seedling is ensured for some more years. Every seedling organ has a time limit for sustaining the resources; however, the time limit will vary from habitat to habitat. At the final stage of establishment of a young tree none of the seed and seedling organs survive or exist in traces. Their role is to structure the sporophyte and make it to survive for longer years. Thus, the seed germination process in Palmyra palm is a well evolved and highly structured phenomenon to face any kind of disturbances till establishing a strong juvenile tree.

Conclusion

The present study on Palmyra Palm – *Borassus flabellifer* L. is focused upon understanding the germination process, anatomy of seedling organs, resource mobilization and biomass allocation of cotyledonary sheath. The structure of endosperm, haustorium, and morphology of the seedling organs of *B. flabellifer* are described with developmental phases and storage of starch. The study also extended to trace some evidences of biochemical changes that take place during the development of seedling. Since palmyra palm is a monocotylon, seed embryo and related seedling organs have been redefined with suitable terminologies applicable to monocotyledons. The digestion zone between endosperm and haustorium has been found to have simple sugars, amino acids, proteins and very minute level of lipids. Histochemical tests have confirmed the presence of simple sugars and proteins at digestion zone as well as inside the haustorial tissues. Simple sugars are abundant in the digested zone and haustorial tissues. Qualitative test for carbohydrates also confirmed the abundant presence of the same in haustorium.

Resource mobilization is also confirmed by anatomical observations in cotyledonary sheaths. The anatomical features of cotyledonary sheath have revealed the abundant presence of sugars. The cotyledonary sheath transports nutrients such as simple sugars, proteins to the developing cataphyll for nourishment, and these findings open new insights to the field of Palmyra Palm seed biology.

Conflicts of interest

Authors declare no competing interests.

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