Seed microstructures of the Andaman padauk (*Pterocarpus dalbergoides*)

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Andaman padauk (*Pterocarpus dalbergioides* Roxb.) is an important endemic tree species of the Andaman Islands, India, belonging to the family Fabaceae. We studied the seed microstructures which provide information on the physiological features of germination (i.e. factors responsible for the resumption of growth of embryo). Seed anatomy/morphostructures revealed information on its germination physiology (for *ex situ* conservation), dormancy barriers and further taxonomical classification. The seed samples were examined under a scanning electron microscope (SEM) and Fourier-transform infrared spectroscopy (FTIR) was used to study the chemical composition of the seeds. In the SEM images, seed thickness of the parenchyma layer was about 53.16 μm, while that of the osteosclereids (OH) and macrosclereids (MH) layers was 23.95 and 84.68 μm respectively. The size of OS cells had a length of 25.81–34.56 μm; diameter 12.14–16.5 μm and width 7.97–12.60 μm. FTIR spectra of padauk seed assignments were used to determine the probable chromophoric groups that are likely to be present in the seeds. Various functional groups such as N–H, O–H, C–H, C=O, C–O were identified in the seeds. The results indicate the presence of carbohydrates, amino acids, amides, esters, ethers, phenols, proteins and fats in the padauk seeds. These observations would be helpful in correlating several aspects of seed germination/dormancy physiology of this endemic species, which has applications in *ex situ* conservation.

Keywords: Chromophoric groups, *Pterocarpus dalbergioides* Roxb, seed microstructures.

ANDAMAN padauk (*Pterocarpus dalbergioides* Roxb.), also called the East Indian mahogany, is an endemic tree belonging to the family Fabaceae. It is a large deciduous tree, mostly found in sedimentary soil consisting of sandstone and conglomerate in natural habitats. The mature trees have tall stature of about 80–127 ft, and buttress roots, and the species has been designated as the state tree of Andaman and Nicobar Islands. The trees are characterized by alternate compound leaves with alternate leaflets, yellow-coloured flowers in racemes (panicled), and one- or two-seeded, indehiscent, flat, orbicular pods, with seeds surrounded by a wide circular wing. Andaman padauk wood is used for innumerable purposes; it varies in colour and is highly valued[1]. It is used for ornamental and decorative work and has emerged as the principal timber tree of the Andamans, triggering economic revolution in the Island[2]. However, padauk tree population is affected by logging operations on the one hand and poor regeneration status on the other. The tree has been declared as a reserved tree due to the threat of extinction with poor natural seed germination status. FAO has also prioritized the conservation efforts considering the limited pattern of distribution and economic importance of the species[3].

With the need to conserve this species, the present study was undertaken to gain deeper insights on seed anatomy. Examining the seed anatomy/morphostructures is essential to know about its germination physiology (for *ex situ* conservation), dormancy barriers and further taxonomical classification. The tree profusely produces fruits with less of mature seeds and more ill-filled/false seeds in the fruit (samara); 1452 fruits go to make one kilogram. Under *ex situ* conditions, the seed germination time was 12 to 15 days with maximum of 45% germination and 20% plant recovery[4].

A study of seed microstructures provides information on the physiological features of seed germination (i.e. factors responsible for the resumption of growth of embryo), apart from favourable environmental conditions[5]. Understanding the seed structure, composition and adaptive traits of endemic species will be helpful in strengthening the conservation efforts. Hence, the present study was undertaken to demarcate the seed micromorphology of *Pterocarpus* species endemic to the Andaman Islands. The data obtained will be used to correlate several aspects of seed germination/dormancy physiology. This study will further help in identifying seeds of six other *Pterocarpus* species and their phylogenetic differences.

The fully mature and dry fruit was collected from randomly selected padauk trees and mechanically dehisced to remove the seeds. The ectocarp (testa) and endocarp (endosperm) were also separated mechanically. At the time of sample analysis, samples were placed on a double-stick carbon tape affixed to a carbon stub, and vacuum-desiccated for 2 min to remove excess moisture from the stub. The seed sample was examined under a Scanning Electron Microscope (FEI SEM, Quanta 250) using an LFD detector. The SEM was operated at 5–20 kV with a working distance of 13–14 μm. Energy-dispersive X-ray spectroscopy (EDX) analysis was performed in the true seed. The facility at SRM University, Chennai, was used for the study.

Fourier-transform infrared spectroscopy (FTIR) model Jasco FT/IR 6800 was used to analyse the chemical composition in the true seed. The IR spectrum of a compound records the absorption bands of specific functional groups of molecules. Seeds were dried, powdered and sieved before analysis. After loading the sample, it was analysed in

attenuated total reflectance (ATR/FTIR) and spectral data were recorded at 64 scans/s with a resolution of 4 cm^{-1} and the spectral range was from 400 to 4000 cm. The facility at Tamil Nadu Agricultural University, Coimbatore, was used for the study.

The fruits of Andaman padauk (*Pterocarpus* sp.) are brown to black in colour, winged, compressed or flattened, stipitate, replum invisible with elevated outer protective seed chambers; reticulately veined and bilocular. The length of the fruit ranges from 4.5 to 7.6 cm and width from 4.8 to 7.7 cm; 100 seed weight is 5.47 g. The seeds are reniform, *D*-shaped or sickle-shaped; light to reddish-brown or tan; smooth testa not adhered to endocarp; hilum and micropyle visible and radicular lobe present (Figure 1). These descriptions on the fruit/seed features match with the fundamental patterns of Fabaceae genera[6].

SEM images of the seed exomorphic features reveal interesting details related to the origin of Fabaceae. The true seed coat tissue components show the presence of a well-differentiated hilum, a micropylar region and continuous cuticle layer (Figure 2 *a*), depicting the common characteristics of Fabaceae family seed coat structures[7], although some intra- and inter-specific variations may exist within the *Pterocarpus* species[7,8]. The hilum o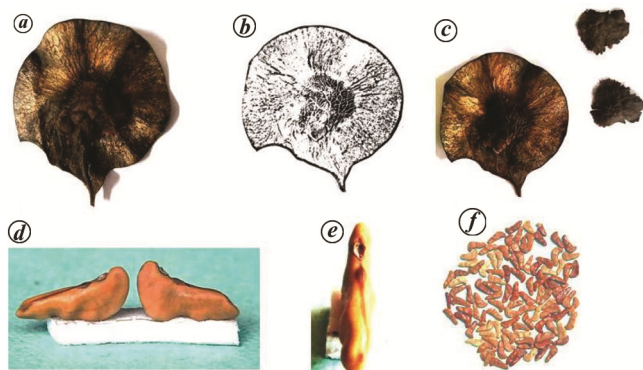f the seed is a distinct region, oval or round in shape with rough layers and a central fissure. This is also called the hilar groove, which is a commonly observed structure of the legume and acts like a valve regulating moisture levels[7,9,10]. Under low relative humidity (RH), the hilar fissure opens up and vice versa under high RH levels[11]. Functionally, under the influence of external and internal factors the hilum and the micropylar region perform water absorption and opening. The magnified cuticular layer has cracks and pits with waxy deposition (Figure 2 *b*). This is often the primary barrier to imbibition in the seeds[12–14].

Fully mature, dry seeds were taken for the examination, cut transversely. Figure 3 shows a thin, delicate, undulated epidermal cell layer (EL). The EL forms a single layer above the sclereid layers. The radially elongated and tightly packed cells are referred to as macrosclereids (MS) or palisade cells. The length of the MS may vary among different members of the Fabaceae family, and also within different *Pterocarpus* species[15–18]. This difference is also regarded to be an environmental variation[19].

The next consecutive layer of the seed coat depicts interconnected parenchymatous cells (PCs) with intercellular spaces, in which are embedded the dumbbell-shaped cells called osteosclereids (OS)[11]. They are also reported to have air-filled intercellular spaces. The MS and OS cell arrangement in the testa architecture has been correlated with the dormancy factors causing impermeability to water Jerard. The thickness of the parenchyma layer is

Figure 1. *a–c*, Padauk fruit with seed chambers and reticulate veins. *d–f*, True seeds reniform shaped, varying in colour from light brown to tan; hilum and radicular lobe present.
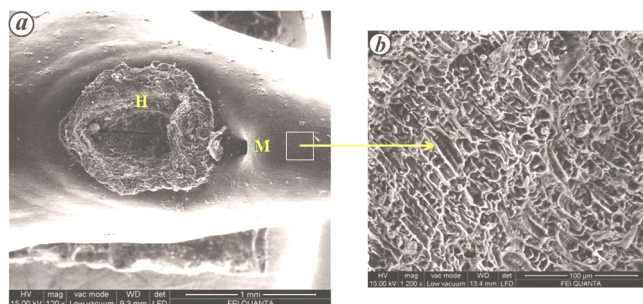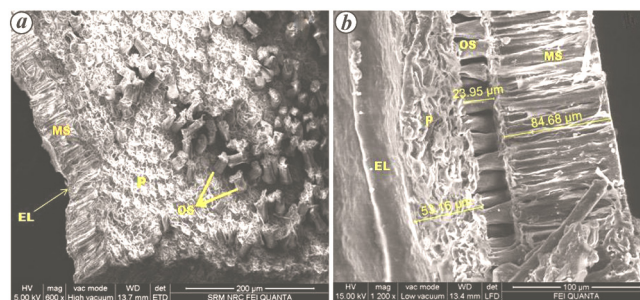

Figure 2. *a*, True seed depicting hilum (H) and micropyle region (M). *b*, Magnified surface structure.

Figure 3. SEM images of a mature dry padauk seed depicting (*a*) outer testa and (*b*) magnified cross-sectional view of the seed.
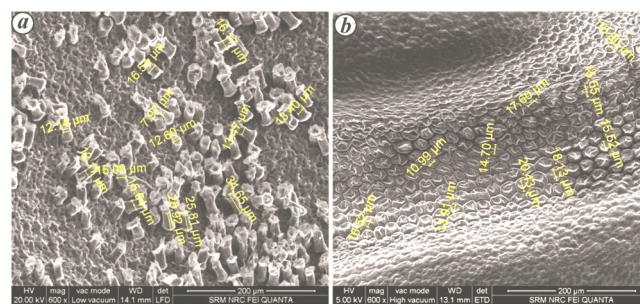

Figure 4. SEM images showing size of (*a*) osteosclereid cells on the seed exomorph and (*b*) endosperm starch granules.

around 53.16 µm, while that of the OS and MS layers is 23.95 and 84.68 µm respectively. The size of OS cells has a length of 25.81–34.56 µm, diameter 12.14–16.5 µm and width 7.97–12.60 µm (Figure 4). The PCs consist of vascular systems that provide nutrition to the growing embryo. Hence, the seed coat pattern in the Andaman padauk provides mechanical protection as well as physical dormancy. The seed characteristics of Fabaceae share some common structural features like the EL differentiation into MS, OS and PC disintegration at maturity which play a key role in hardening of the seed coat[14,20,21]. These developmental events support various physiological functions like embryogenesis germination, assimilate partitioning, metabolic conversion, etc. The interconnected PCs favour starch accumulation transiently to transfer nutrients to the zygote. In general, the MS layer has a vital role in determining the hardness of the seed coat[22].

The inside of the seed encompassing the endosperm supplies nutrients to the developing embryo. SEM image shows starch granules (SGs) with intercellular spaces that act as store reserves for further metabolic interconversion (Figure 5). The interlocular spaces observed could be due to the shrinkage of SGs in the fully mature and dry seeds. The endosperm cell layer has a wavy or fold appearance owing to the sickle-shaped cells of the Andaman padauk seed.
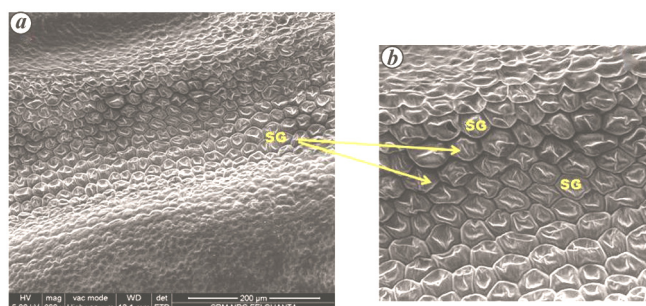
EDX characterization technique was used to determine the elemental composition. EDX analysis indicates the count of a particular element at the point of interest. Red arrow in Figure 6 indicates the point of analysis. Mass % reveals the amount of a particular element (Figure 6).

Figure 7 shows the FTIR spectrum of padauk seeds. Table 1 shows the visual intensity and assignments for various absorption bands. The assignments were used to determine the probable chromophoric groups that are likely to be present in the seeds.

The strong absorption band around 3300–3280 cm^{-1} found in the spectra of seed samples is due to the presence of O–H stretching of water molecules[23–25]. The strong absorption bands observed at 2923 and 2864 cm^{-1} belong to vibration of C–H asymmetric and symmetric stretching vibration of mainly lipids and proteins[24,26–29]. This also corresponds to symmetric stretching and presence of aliphatic (–CH) groups in these compounds[30,31]. The strong IR band at 1660–1600 cm^{-1} is characteristic of C=O stretching of ketones, and the stretching from 1620 to 1580 cm^{-1} is due to the presence of N–H deformation of amino acids[31–35]. The weak absorption band observed

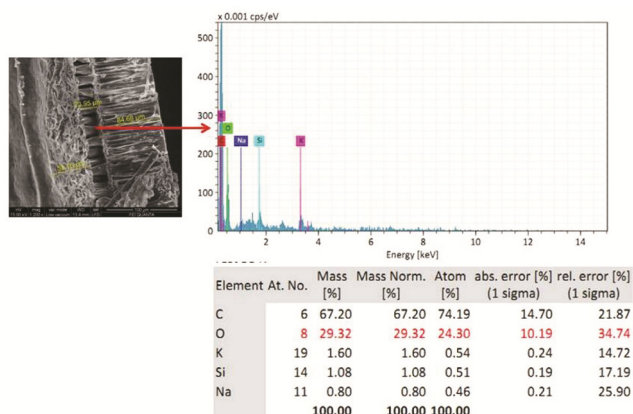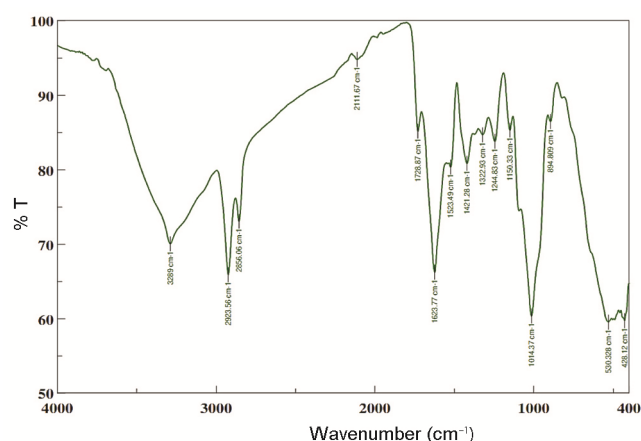**Figure 5.** SEM images of (*a*) the seed endosperm layer and (*b*) starch granules.

Figure 6. EDX analysis of the true seed depicting elemental composition; red arrow indicates the point of analysis.

Figure 7. FTIR spectrum of padauk seeds.

Table 1. FTIR absorption frequencies, visual intensity and assignments in the padauk seeds

Absorption frequency (cm^{-1})	Visual intensity	Assignments
3289	Strong	O–H stretching
2923	Medium	C–H asymmetric stretching
2856	Strong	C–H symmetric stretching
1623	Strong	C=O stretching
1523	Strong	N–H deformation
1421	Weak	O–H deformation
1322	Strong	C–O stretching
1244	Strong	C–O stretching
1150	Strong	C–O stretching
1014	Strong	C–O stretching
894	Medium	O–H deformation
530	Medium weak	N–H deformation

at 1421 cm^{-1} belongs to O–H deformation. The strong absorption bands in the range 1020–1340 cm^{-1} are due to C–O stretching vibration of esters and phenols[31,33,35,36]. The medium and medium–weak absorption bands at 894 and 530 cm^{-1} are due to out-of-plane O–H and N–H deformation respectively. Hence, in the present study, various functional groups such as N–H, O–H, C–H, C=O, C–O have been identified in the seeds. This shows the presence of carbohydrates, amino acids, amides, esters, ethers, phenols, proteins and fats in the padauk seeds. The data obtained would be highly significant to correlate several aspects of seed germination/dormancy physiology. The genera *Pterocarpus* has four species in India that have commercial timber value. They are *Pterocarpus santalinus*, *P. indicus*, *P. dalbergioides* and *P. marsupium*. The distribution, growth and establishment of these species are known to be confined to different geographical localities. Among these, *P. dalbergioides* occurs only in the Andaman Islands and has been a well-utilized timber species in the Islands. Natural regeneration of padauk has been reported to be low at different locations. The population of Andaman padauk has been reported to be declining over the years due to felling and also due to change in weather parameters. Considering the importance of this endemic species in timber production and its environmental services to the fragile Island ecosystem, there is a need to strengthen the expansion of area under padauk. The understanding of seed composition, physiological traits and morphology of seeds would help in formulating strategies for afforestation programmes. This study will further help in differentiating the seeds of six other *Pterocarpus* species and their phylogenetic differences. Further comparison of seed parameters of other species would give more insight into the varied occurrence of *Pterocarpus* species at different locations based on their adaptive traits.

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