Taxonomy, Distribution and Economic Importance of *Xylocarpus* species at Carey Island – The Heritage Island of Malaysia

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Abstract

The genus *Xylocarpus*, belonging to the family Meliaceae is distributed in the tropics including mangrove habitat from Africa to Australia, Malaysia and India (Ridley, 1922). It is usually associated with *Avicennia*, *Excoecaria*, *Acanthus*, *Rhizophora*, *Bruguiera*, *Sonneratia*, *Nypa* and *Ceriops*. There are three species of *Xylocarpus* in Malaysia namely, *Xylocarpus granatum*, *X. mollucensis* and *X. rumphii*. They are considered important endangered mangrove species in Malaysia. *X. granatum*, commonly known as *nyireh bunga*, is important economically for wood carving. The inner bark is a source of dye for tanning, the oil from seeds is used for grooming hair, the fruits and seeds are used to treat diarrhea, and a bark decoction for cholera. It has been mentioned as the best and most beautiful cabinet wood. Its fine, glossy texture is suitable for furniture (Burkill, 1966; Primavera *et. al*, 2004). However, the population of the species is dwindling hence there is an urgent need to conserve the species.To date, there is no record on detailed morphological study of the species therefore it is one of the aims of the study to investigate the morphological characteristics of the species. Anatomical studies on the leaf were also carried out. Scanning electron microscope study revealed the presence of sunken, anomocytic-type stomata on the abaxial surface of the leaf. Results from anatomical studies showed the presence of thick cuticle on both abaxial and adaxial surface of the leaf. Sunken stomata and thick cuticle are adaptations of mangrove species to reduce transpiration. Tannin cells were also observed in the leaf lamina. Regeneration of this species from tissue culture had been attempted, however, only callus formation was observed.

INTRODUCTION

Carey Island is situated in the North-West of Morib, Selangor, across the sea from Port Klang, Malaysia (figs 1&2). It is a huge island with a total area of 15,000 ha and comprised largely of palm oil estate belonging to Sime Darby Plantation, a leading plantation development group in Malaysia. It is an initial settlement area for the Mah Meri, one of the aborigine tribe of Malaysia. The tribe is renowed for their woodcarving, using the wood of *Xylocarpus* species. On Carey Island, the development is geared towards a model region where past and special attributes of the landscape are coupled into the unfolding development with special emphasis on

conserving as many natural and biodiversity perspective as possible. University of Malaya and Sime Darby have formed a collaborative relationship for specially addressing research and development that could be undertaken with reference to conservation and development of biodiversity and natural resources on Carey Island. Floristic studies of the island has been conducted. This paper will focus on two *Xylocarpus* species found on Carey Island i.e. *Xylocarpus granatum* and *Xylocarpus moluccensis* which are considered as important endangered mangrove species in Malaysia. Propagation of the species through tissue culture as an approach for conservation will also be discussed.

MATERIALS AND METHODS

In the present study, morphological study was carried on *Xylocarpus granatum* and *Xylocarpus moluccensis* while anatomical and tissue culture studies were carried out on *Xylocarpus granatum*. To date, there is no record on detailed morphological study of the species, therefore it is one of the aims of the study to investigate the morphological characteristics of the species. Morphological studies were conducted using light and scanning electron microscope. Morphology of the intact leaves were also investigated since no such work have been done before. Anatomical studies on the leaf were also carried out and the technique adopted was from Johansen (1940). Regeneration of this species from tissue culture had also been attempted as an approach for conservation. Murashige and Skoog (1962) media supplemented with hormones was used in tissue culture studies.

RESULTS

Xylocarpus granatum Koen. is commonly known as Nyireh bunga (Malaysia); tabigi (Philippines); tabun kao (Thailand). It is a medium tree up to 17 m. tall. It has compound leaves, arranged oppositely, obovate in shaped with entire margin. The leaf is 12 (7-19) cm. long and 6 (4-9) cm wide. The leaf apex is round to emarginated, base acute, the adaxial surface smooth, dark green, abaxial surface smooth and light green. The smooth, light brown to greenish outer bark flakes off and the inner bark is red. The inflorescence is paniculate, usually axillary with 4 white petals and 4 yellowish green calyx. The fruit is a big, brown globose, like cannon ball or bowling ball with 10-12 irregularly shaped seeds (Figs. 3-6). It is distributed in the tropics all round the world, from East Africa to the Pacific Islands. In Malaysia, it is found along estuarine rivers and tidal creeks, whose low buttresses extend as distinctive, snake-like plank roots. Also found in higher intertidal, inner mangroves as part of a mixed community that includes, among others *X. moluccensis*, *Ceriops decandra* and *Bruguiera cylindrica.* The inner bark is a source of dye for tanning. Oil from seed is used for lamps and for grooming hair. The fruits and seeds are used to treat diarrhea and a bark decoction for cholera. It has been described as the best and most beautiful cabinet wood. Its fine, glossy texture is suitable for furniture. It was used for poles, railroad tiles, posts and beams.

Xylocarpus moluccensis (Lam.) M. Roem. is commonly known as Nyireh batu (Malaysia); piagao (Philippines); taban (Thailand). It is a small tree, 3-10 m tall. It has compound leaves, arranged oppositely, elliptic in shaped with entire margin. The leaf is 8 (5-12) cm. long and 4 (2.5) cm wide. The leaf apex and base is acute, the adaxial surface smooth, green, abaxial surface smooth and light green. The species is deciduous; the leaves turn golden brown to red then drop; the new leaves appear together with the short-lived flowers. The outer bark is rough, dark brown and fissured. It has peg- or cone-shaped pneumatophores (figs.7). The

inflorescence is paniculate, usually axillary with 4 white petals and 4 pale yellow- green calyx. The fruit is light green, like a small cannon ball. It is distributed from the Mascarene Islands to the islands of the Pacific. In Malaysia, it is found on the firm substrate of back mangroves rarely appearing along the edges of rivers or creeks. Their low-salinity habitats overlaps with those of *X. granatum.* Seeds were used for insect bites, diarrhea and as astringent, the fruits for diarrhea, and the bark as astringent. The wood has been used as poles, railroad ties, posts, beams and for interior finish, musical instruments and high grade furniture.

Scanning electron microscope study revealed the presence of sunken, anomocytic-type stomata on the abaxial surface of the leaf. More stomata were observed on the abaxial surface of matured leaf compared to the abaxial surface of young leaf (Fig. 8&9). Studies on both young and matured intact leaves also showed the presence of undulating anticlinal walls on the abaxial and adaxial surfaces. Undulating walls may be due to the pressure exerted on the cells during differentiation phase in leaf morphogenesis. The formation of undulate walls may also be affected by environmental factors (Esau, 1977).

Results from anatomical studies showed the presence of thick cuticle on both abaxial and adaxial surfaces of the leaves. Sunken stomata and thick cuticle are adaptations of mangrove species to reduce transpiration. One to two layers of epidermal cells were observed on both the abaxial and adaxial surfaces of the leaf. Palisade cells are biseriate, long, large and close together while the vascular bundle is arc-shaped and collateral (Fig. 10). Tannin cells were also observed in the leaf lamina and this needs further investigation.

Regeneration of this species from tissue culture proved to be difficult, thus far only callus formation was observed. Formation of callus from leaf segments, young stems and flower buds were observed after three weeks being cultured on MS medium supplemented with 2.5 mg/l 2,4, Dichlorophenoxy acetic acid (2,4-D). A range of concentration from 0.5 to 20 mg/l of 2,4-D were tested. MS medium supplemented with 10 mg/l 2,4-D and 10 mg/l Naphthalene acetic acid (NAA) managed to produce 87.5% callus after 14 days in culture when leaf explants were used. It was observed that more callus was formed when the cultures were maintained at 25°C in the dark. At pH 4.0, the callus seemed to form faster and larger amount of callus were formed compared to other pH media. The morphology of the callus is shiny, creamy and some nodular structures were observed. The addition of 0.1 g/l sodium chloride (NaCl) to MS medium produced 70% callus. The range of NaCl tested was from 0.1g/l to 7.0g/l. However, the results obtained were not significantly different compared to at concentration of 0.1g/l. The callus produced was analysed under microscopes and double staining method was used to check for any formation of embryogenic callus. A few callus cells were found to be in the globular stage of somatic embryogenesis but failed to develop further to other stages such as heart or torpedo shaped embryos (Fig. 11). Work is in progress to induce somatic embryogenesis in this species.

DISCUSSION

Leaf feature such as surface sculpturing is found to be useful to aid species identification and elucidation. The combination of high magnification, high resolution and great depth of focus of the scanning electron microscope has enabled us to observed and study features of the leaf surface in great detail. Leaf epidermal and cuticular characters are often of real value in

taxonomic and phylogenetic investigations (Dilcher, 1974, Stace, 1966). Although information regarding the systematic anatomy of the epidermis or cuticle is quite impressive in some groups, such as Gramineae and gymnosperms, relatively little work has been attempted in the dicotyledons and other monocotyledons. Many surveys appear to have been undertaken on xeromorphic plants (e.g. Lauraceae, Magnoliales), which have a thick cuticle and show the epidermal pattern clearly. Stace (1966) managed to separate easily three groups of mangroves by means of epidermal characters alone. The size of the epidermal cells is not assumed to be a reliable taxonomic character since it has been emphasized by previous workers that the variability of the epidermal cell size is correlated with the age of the leaf, genetic variation and environment (Yapp, 1912, Stace, 1965). In the present study, the mesophyll showed a clear differentiation between a well-formed palisade and the adjacent spongy layer. Many studies indicate that the degree of mesophyll differentiation is highly dependent on the degree of exposure to the sun (Hanson, 1917, Ryder, 1954). They proposed that leaves growing in sunshine have more palisade layers compared to leaves in shade, and palisade cells are long, large and close together. These characters have been observed in the present study. Leaves in heavy shade have short, thin and loosely arranged palisade cells. Salt glands were not observed in the leaves of the species studied and the present result agreed with the results obtained by Das (2002). This indicate that different mangrove species give a different respond to salinity (Scholander,1968).

Regeneration of this species using tissue culture and other biotechnological methods proved to be vital for conservation of these rare and endangered species. However, they are very recalcitrant to tissue culture system and thus far only callus formation was obtained from very limited explants such as leaf, stem and flower buds. Formation of callus from leaf segments, young stems and flower buds were observed after three weeks being cultured on MS medium supplemented with 2.5 mg/l 2,4, Dichlorophenoxyacetic acid (2,4-D). A range of concentration from 0.5 to 20 mg/l of 2,4,D were used. MS medium supplemented with 10 mg/l 2,4-D and 10 mg/l Naphthalene acetic acid (NAA) managed to produce 87.5% callus after 14 days in culture from leaf explants. Other than MS, DKW medium was also tested but not effective. It was observed that more callus was formed when the cultures were maintained in the dark. At pH 4.0, the callus seemed to form faster and more callus were formed compared to other pH media. The morphology of the callus is shiny, creamy and some nodular structures were observed. The addition of 0.1 g/l sodium chloride (NaCl) to MS medium produced 70% callus. The callus produced was analysed under microscopes and double staining method was used to check for any formation of embryogenic callus. A few callus cells were found to be in the globular stage of somatic embryogenesis but failed to develop into other stages such as heart or torpedo shaped embryos, even when the cultures were transferred to cell suspension culture. Work is in progress to induce somatic embryogenesis in this species. Since very limited tissue culture work was done on *Xylocarpus* spp. it is very difficult to compare with other research. Various media were tried such as DKW, etc but not effective. Different concentration of sodium chloride (0.1-7.0 g/l) were tested but only 0.1g/l proved to be the most effective. Various pH media (4-7) were tested, with pH 5.8 as control and it was found that pH 4 was the best so far. Active tissue culture research of these species is still ongoing in our lab.

ACKNOWLEDGEMENTS

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Figures

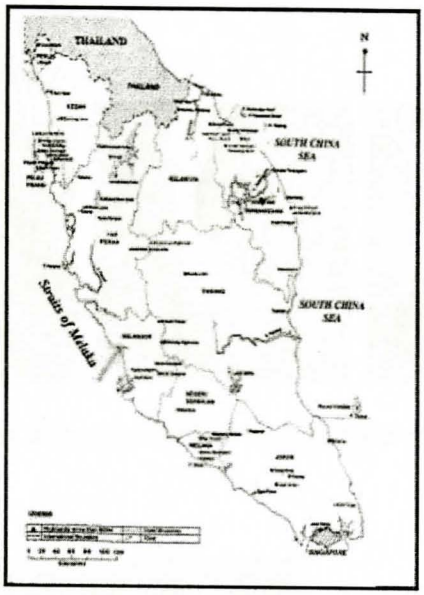

Fig. 1. Map of Peninsular Malaysia

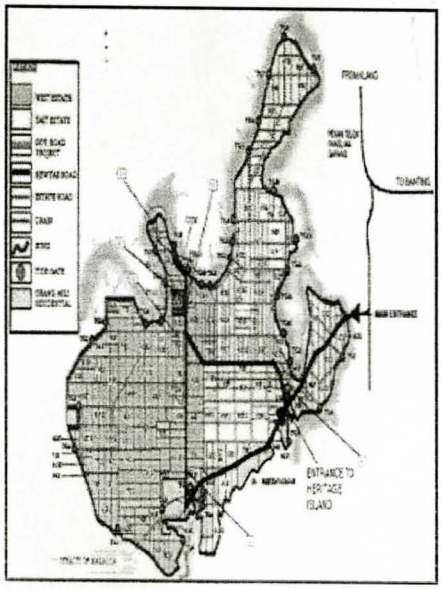

Fig. 2. Map of Carey Island, Malaysia

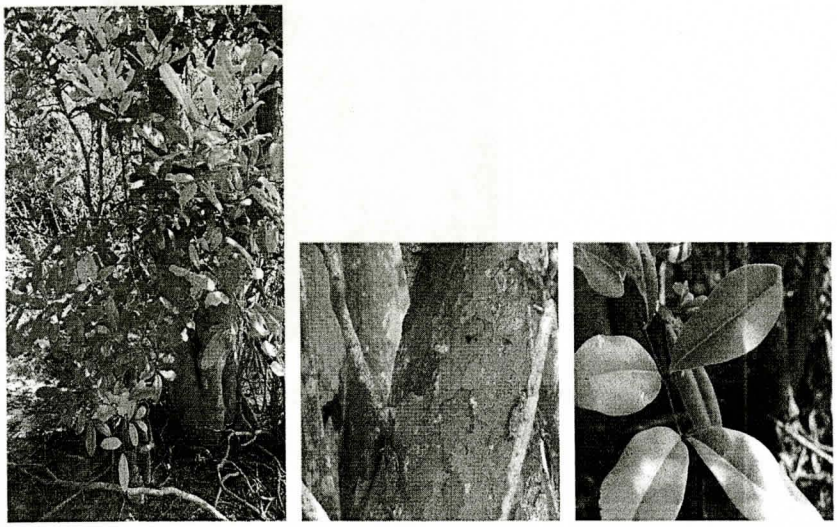

Fig. 3. Habit, leaf arrangement and bark surface of *Xylocarpus granatum*.

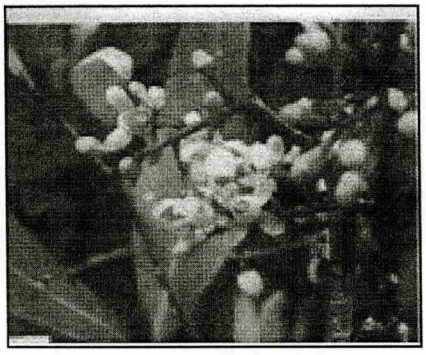

Fig. 4. Flowers borne on inflorescence

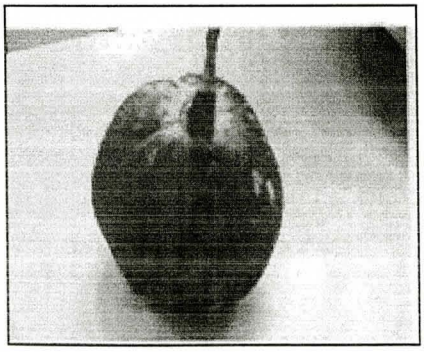

Fig. 5. Shape of fruit

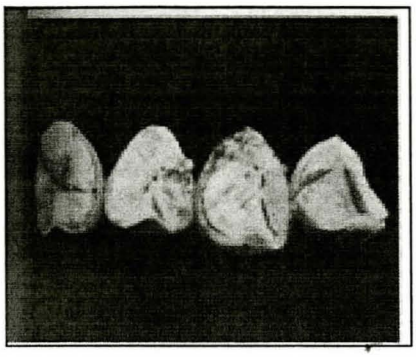

Fig. 6. Seeds of *Xylocarpus granatum*

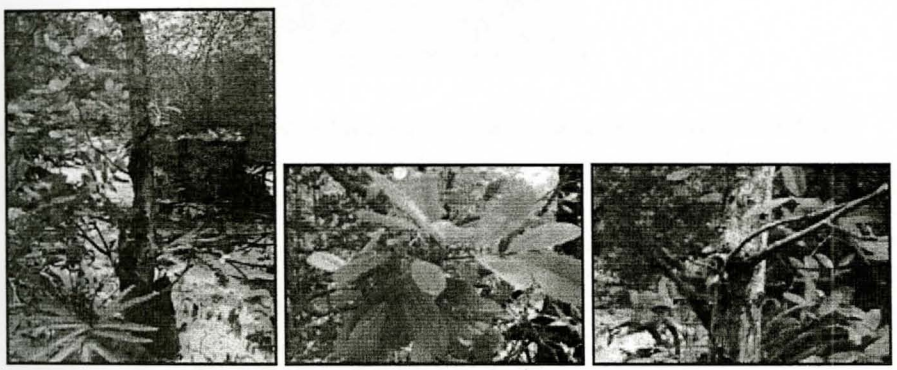

Fig. 7. Habit, leaf arrangement and bark surface of *Xylocarpus moluccensis*

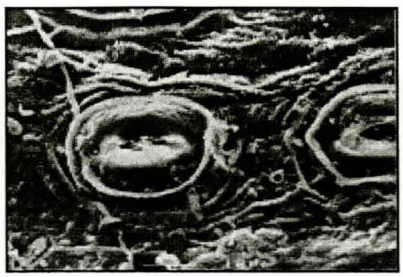

Fig. 8. Anomocyctic, sunken stomata on abaxial surface of mature intact leaf

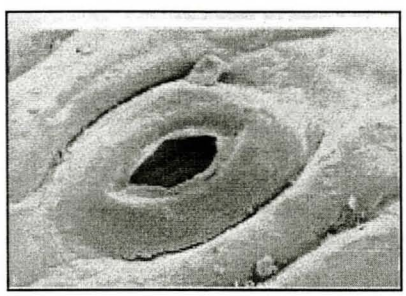

Fig. 9. Anomocyctic, sunken stomata on abaxial surface of young intact leaf

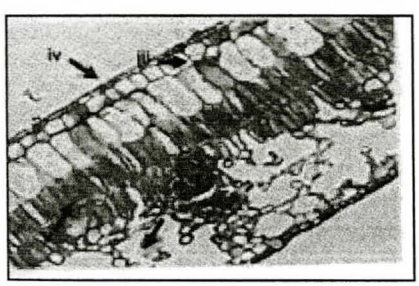

Fig. 10. Cross section of leaf lamina showing (i) vascular bundle (ii) epidermal cells (iii) hypodermis (iv) cuticle layer (v) palisade cells (vi) spongy mesophyll

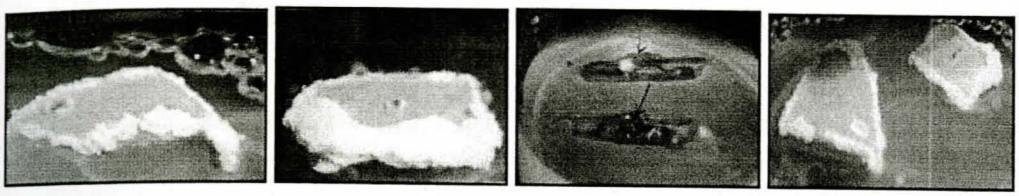

Fig.11. Callus formation of *Xylocarpus granatum* through tissue culture for mass propagation.