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Morphological, Anatomical and Tissue Culture Studies on *Xylocarpus granatum*

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ABSTRACT

Xylocarpus species are important endangered mangrove species in Malaysia. One of the economic importance of the species, particularly *Xylocarpus granatum* is for wood carving. Hence, there is an urgent need to conserve the species. In the present study, some morphological, 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. Anatomical studies on the leaf and primary stem 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 surfaces 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 and primary stem and this needs further investigation. Regeneration of this species from tissue culture had been attempted, however, 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 2.5mg/l 2,4, Dichlorophenoxyacetic acid (2,4-D). MS medium supplemented with 10 mg/l 2,4-D and 10mg/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 calli were formed when the cultures were maintained in the dark. At pH 4.0, the cultures seemed to form faster and more calli were formed. The addition of 0.1g/l sodium chloride (NaCl) to MS medium produced 70% callus. The callus produced was analysed to see whether they are embryogenic or non-embryogenic. A few callus cells were found to be in the globular stage of embryogenesis but failed to develop further. Work is in progress to induce somatic embryogenesis in this species.

Key words: Morphology, anatomy, *Xylocarpus granatum*, tissue culture, callus, hormones.

INTRODUCTION

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* spp., *Excoecaria* spp., *Acanthus* spp., *Rhizophora* spp., *Bruguiera* spp., *Sonneratia* spp., *Nypa* spp. and *Ceriops* spp.. There are three species of *Xylocarpus* in Malaysia namely, *Xylocarpus granatum*, *X. moluccensis* 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.

MATERIALS AND METHODS

In the present study, some morphological, 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 microscopes Anatomical studies on the leaf and primary stem were also carried out. 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) medium supplemented with hormones was used in tissue culture studies.

RESULTS

Morphology

Xylocarpus granatum is a tree of moderate size, reaching up to 25m tall, usually with crooked trunk. It can be differentiated from *X. moluccensis* by the texture of the bark, the size of the fruit and the shape of the leaves. It possesses smooth, peeling bark, pale reddish brown in colour. It has a compound leves, leaflets ovate to elliptic, withering orange red, with round apex and acute base, short and slightly swollen petiole. The flowers are borne on inflorescence, bisexual, sweet-scented, whitish to pinkish in colour with four sepals and eight petals. The ovary is superior, fruit globose, 25-30cm in diameter with six to 12 seeds (Figs. 1A-F).

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 (Figs. 2A and 2B). 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

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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).

Anatomy

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. Cross section of the primary stem showed the presence of a single layer of epidermal cells while the pith is abundant with parenchyma cells. However, vascular bundle is not clearly observed (Fig. 2C). This may be due to the age of the stem where xylem and phloem tissues are still at the early stage of development. Tannin cells were also observed in the leaf lamina and primary stem and this needs further investigation.

Tissue Culture

For tissue culture studies of *Xylocarpus granatum*, various explants were tested including the shoots, young leaves and flower buds of the intact plants, apart from the leaves and young stems obtained from the seedlings grown in the lab. Regeneration of this species from tissue culture proved to be extremely difficult, only callus formation was observed. Various growth regulators such as Gibberellic acid, TDZ, and BAP were supplemented to the media. However, no callus formation or regeneration was achieved. Formation of callus from leaf segments, young stems and flower buds were observed after three weeks being cultured on MS medium supplemented with 2.5mg/l 2,4, Dichloro-phenoxy acetic acid (2,4-D). 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 leaves were used as explants. It was observed that more calli were formed when the cultures were maintained in the dark. The addition of 0.1 g/l sodium chloride (NaCl) to MS medium produced 70% callus. The callus produced was analysed to see whether they are embryogenic or non- embryogenic. A few callus

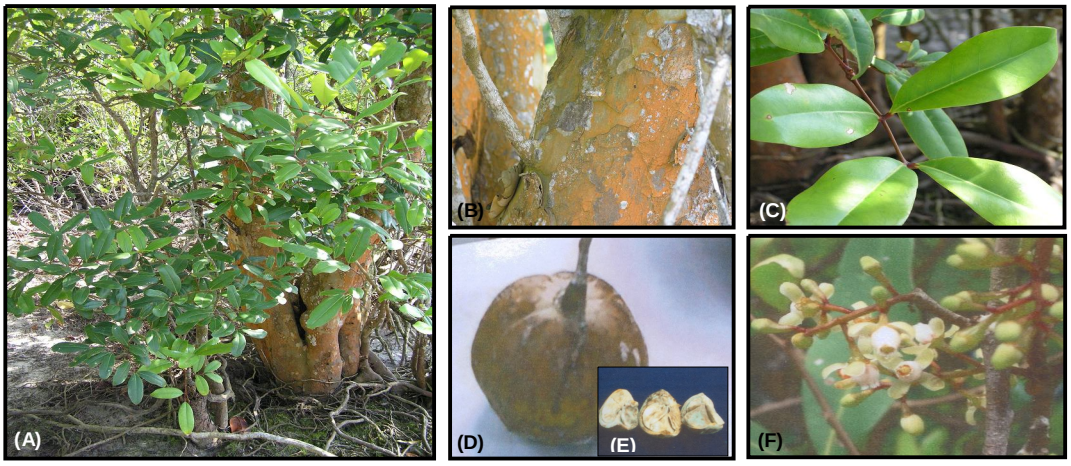

Figure (1): (A) Habit of tree, **(B)** trunk showing smooth, peeling, reddish brown bark, **(C)** leaf shape and arrangement, **(D)** shape of fruit, and **(E)** seeds of *Xylocarpus granatum* , and **(F)** flowers borne on inflorescence.

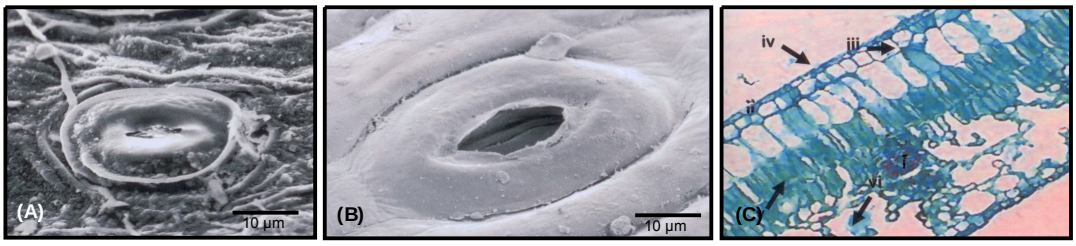

Figure (2): (A) anomocytic, sunken stomata on abaxial surface of mature intact leaf, **(B)** anomocytic, sunken stomata on abaxial surface of young intact leaf, and (**C**) Cross section of leaf lamina showing: (i) vascular bundle (ii) epidermal cells (iii) hypodermis (iv) cuticle layer (v) palisade cells and (vi) spongy mesophyll.

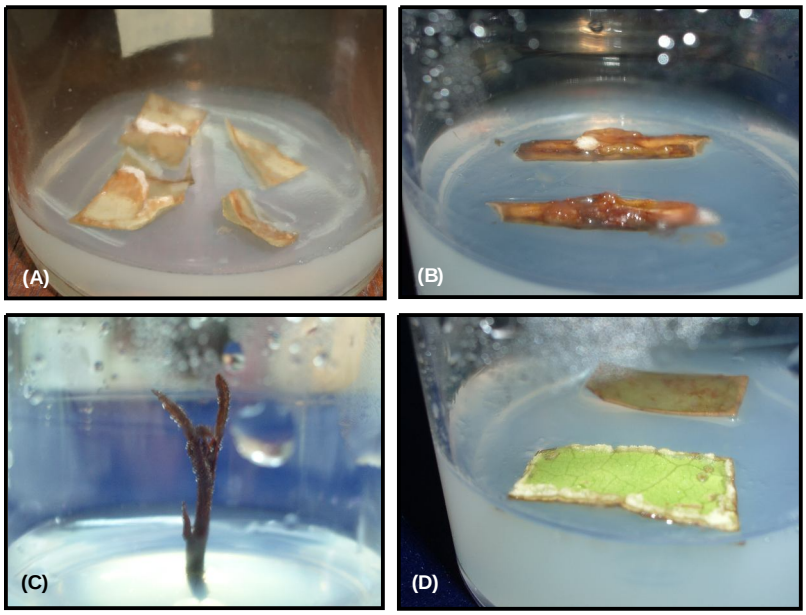

Figure (3): Propagation of *Xylocarpus granatum* through tissue culture.

cells were found to be in the globular stage of embryogenesis but failed to develop further (Fig. 3).
The effect of pH medium was also investigated in the present study and pH of 4 was found to be encouraging for callus growth as compared to 5.8 and 7.0 on the same medium (MS +20 mg/l 2,4-D). The normal pH used commonly was 5.8. 65% of the leaf explants formed callus when the pH of the MS medium was adjusted to 4.0.

DISCUSSION

The combination of high magnification, high resolution and great depth of focus of the scanning electron microscope has enabled us to observe and study features of the leaf surface in more detail. Leaf feature such as surface sculpturing is found to be useful to aid identification and elucidation of species. Leaf epidermal and cuticular characters are often of real value in taxonomic and phylogenetic investigations (Dilcher, 1974; Stace, 1966). Many surveys appear to have been undertaken on plants with xeromorphic (e.g. Lauraceae, Magnoliales), which have a thick cuticle and show the epidermal pattern clearly, usually having a less variable pattern than mesomorphic species. In his studies on mangrove plants, 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 in leaf transection 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). According to them, 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. In leaves in heavy shade the palisade cells tend to become short, thin and loosely arranged. 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).

Thus far, regeneration of this species has never been reported. In the present work, only callus formation was observed. However, work is being aggressively in progress to establish optimum complete plant regeneration medium for this species. Contamination rate by fungi and bacteria proved to be very high when intact explants were used. Once the contamination rate was reduced, callus could also be obtained from intact young leaves, flower buds and axillary buds. The callus obtained was also examined under microscope to detect whether any stages of somatic embryos were formed. However, only cells at globular stage were observed and no further development was seen.

Cultures are sensitive to the pH of the medium (George and Sherrington, 1984). The rapid pH change usually caused by depletion of ammonium in the medium. Naturally, the dissolved calcium of shells and offshore coral make brakish water alkaline. Mangrove soils, however, are neutral to slightly acidic due to the presence of acidic clays. In the present study, pH tested were 4.0, 5.8 and 7.0. For callus formation, pH 4 was the most suitable for *Xylocarpus granatum.*

In previous studies, it was found that addition of NaCl was beneficial to callus growth. In the present work, addition of 0.1g/l NaCl enhanced callus formation and growth. Work is in progress to study the effect of sodium chloride concentrations on callus growth of this species.

As for anatomy and morphological studies of the *in vitro* leaves, no comparison could be made with *in vivo* leaves because plant regeneration was not achieved.

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