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Seed coat sculpturing in *Halophila*

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Abstract—This study furnishes information on external morphology of the seed coats of selected *Halophila*. Fruiting plants of *Halophila beccarii, H. ovalis, H. decipiens, Halophila* sp. were collected from various locations around Malaysia and including *Halophila stipulacea* from Mauritus. Seeds extracted from mature fruits were fixed in 2.5% glutaradehyde. Fixed seeds were washed in 0.1 M Sodium cacodylate buffer at 4°C for 10 minutes. The washing procedure was repeated three times. The seeds were dehydrated in graded acetone series of 35%, 50%, 75%, 95% and 100% followed by in acetone and, dried in a Baltec 030 critical point dryer. After sputter-coating with gold, specimens were examined with a JEOL 6400 scanning electron microscope. *Halophila* seeds are sub-spherical and bluntly beaked at both ends. The *Halophila* seeds from different species showed diverse seed coats morphology with different sculpturing. Seeds of *H. beccarii, Halophila ovalis, H. decipiens* have reticulate coats with variations in microsculpturing within the reticulations. Seed coat of *Halophila* sp. is characteristically verrucose in appearance produced by warts or short peg-like projections. Seeds of *Halophila stipulacea* have reticulate seed coats and the boundaries of the reticulations are joined by peculiar sutures. These diverse seed coats morphology are illustrated by the electron micrographs taken from JEOL 6400 scanning electron microscope.

Key words: seagrasses, surface morphology, seeds, *Halophila*, Malaysia

Introduction

Seagrasses are monocotyledonous aquatic plants with leaves, roots, rhizomes (underground horizontal stems). Seagrasses comprise less than 0.02% of the angiosperm flora, representing a surprisingly small number of species compared with any other group of marine organisms like seaweeds (Hemminga and Duarte, 2000). In contrast to seaweeds, seagrasses flower, develop fruit and produce seeds. In seagrasses, functional seeds are in relation to nutrient storage and germination. According to Kuo and Kirkman (1996) there are three main types of functional seeds in seagrasses in relation to nutrient storage and germination: (i) seeds with hard pericarp e.g. *Zostera, Heterozostera, Heterozostera, Cymodocea, Syringodium, Halodule* or a distinct seed coat e.g. *Halophila*, and a distinct dormancy period, (ii) seeds with a membranous coat and without a distinct dormancy period, e.g. *Enhalus, Thalassia* and *Posidonia* and (iii) seeds which neither have a seed coat nor a dormancy period, e.g. *Thalassodendron* and *Amphibolis*. Seeds of the types (i) and (ii) store starch and protein in the hypocotyledons to be used during germination and seedling development. Seeds of the type (iii) do not store starch and protein but obtain the necessary nutrients for seedling development directly from the parent plants. World wide, the majority of seagrass species are perennial and only few species particularly in the genera *Halophila* have been identified as annual e.g. *H. engelmanii* (McMillan 1986), *H. spinulosa* (Birch 1981), *H. decipiens* (McMillan 1988a), *H. ovalis* (Kuo and Kirkman 1992), *H. tricostata* (Kuo et al. 1993, 1995) and *H. beccarii* (Muta Harah et al. 1999). For the annual populations survival is dependent completely on recruitment of functional seeds and germination (Kuo et al. 1993, Kuo and Kirkman 1995, Muta Harah et al. 1999). Other studies related to seagrass seeds particularly in *Halophila* are those on the seed coat morphology. Reticulate seed coats e.g. in *H. decipiens, H. ovalis*, have been suggested as aiding the seed dispersal (den Hartog 1970, McMillan 1986). In this paper we observed seed coat morphology of four *Halophila*; *H. beccarii, H. decipiens, H. ovalis* and *Halophila* sp. from Malaysia and including *H. stipulacea* from Mauritus under scanning electron microscope. The seeds coat morphology are illustrated and described.

Materials and Methods

Fruiting plants of *H. beccarii, H. decipiens, H. ovalis* and *Halophila* sp. were collected from seagrass meadows of

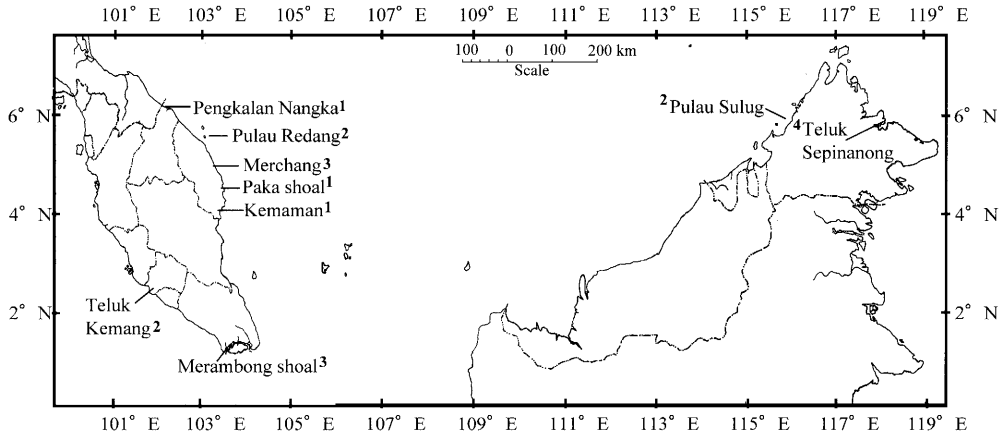

Fig. 1. The study locations: Peninsular Malaysia-Teluk Kemang, Negri Sembilan; Merambong shoal, Johore; Kemaman, Terengganu; Paka shoal, Terengganu, Merchang, Terengganu; Pulau Redang, Terengganu; Pengkalan Nangka, Kelantan; Sabah, East Malaysia-Pulau Sulug, Teluk Sepinanong. 1-*Halophila beccarii*, 2-*H. decipiens*, 3-*H. ovalis*, 4-*Halophila* sp.

Teluk Kemang, Negri Sembilan; Merambong shoal, Johore; Kemaman, Terengganu; Paka shoal, Terengganu, Merchang, Terengganu; Pulau Redang, Terengganu; Pengkalan Nangka, Kelantan; Pulau Sulug, Sabah and Teluk Sepinanong, Sabah (Fig. 1) and Poste La Fayette, Mauritius. Seeds extracted from mature fruits were fixed in 2.5% glutaradehyde. Fixed seeds were then washed in 0.1 M sodium cacodylate buffer at 4°C for 10 minutes. The washing procedure was then repeated three times. The seeds were then dehydrated in graded alcohol series of 35%, 50%, 75%, 95% and 100% followed by in acetone (Glauert, 1975) and, dried in a Baltec 030 critical-point dryer. After sputter-coating with gold, specimens were examined with a JEOL 6400 scanning electron microscope.

Observations and discussion

Halophila seeds are sub-globose and bluntly beaked at both ends. Seeds of *Halophila* exhibit diverse sculpturing morphology. With respect to gross morphology, *Halophila beccarii*, *H. decipiens*, *H. ovalis* have reticulate testa or seed coats. Detailed examination under scanning electron microscope indicated that seed coat of each *Halophila* species have distinct patterns or characters. The seed coat surface *H. beccarii* has reticulation with a pale short brown peg (appearing as brown dot as observed under light microscope) in the center of the reticulation. The surface within the reticulation is smooth (Fig. 2a–d). The seed coat of *H. decipiens* has hexagonal reticulation without peg at the center and rough within the reticulation (Fig. 3a–d). In *H. ovalis*, the surface of the seed covering has a distinct isometric or rectangular reticulation and some microsculptures within the reticulation (Fig. 4a–d). The seed of *Halophila* sp. is sub-globose in shape. The seed coat is without reticulation but verrucose in appearance sculptured with numerous small blunt projections (Fig. 5a–b). *Halophila stipulacea* seed is also sub-globular with reticulate seed coat surface. The boundary of the reticulations has a peculiar fish bones sutures (Fig. 6a–b).

Seed coat reticulations have been observed in other *Halophila* from other regions of the world; *H. beccarii* (Parthasarathy et al. 1988), *H. engelmannii* (McMillan 1986), *H. decipiens* (McMillan 1988b), *H. ovalis* (den Hartog 1970; Kuo and Kirkman 1992) and *H. tricostata* (Kuo et al. 1993). It has been reported that the reticulate seed coat probably facilitates floating by entrapment of air bubbles and dispersal of seeds. The seed coat of *Halophila spinulosa* is characteristically sculptured with numerous long small peg-like projections (Birch 1981). The peg-like projections in *H. spinulosa* and *Halophila* sp. (in our present observation) maybe less conducive to floating. In addition Birch (1981) reported that capsules or seed coats of *H. spinulosa* seeds floated but not the seeds. In *H. beccarii*, although the seed coat has reticulation, the seed is negative buoyant (Muta Harah et al. 1999) and may not promote floating and may not be conducive to the wide dispersal of seed. Irrespective of species, the seed coat sculpturing has some functional significance in providing surface friction against the substratum whereby the seed coat loosened around the seed before emergence of the coiled cotyledon (Birch 1981) probably for the initiation of germination. Additional studies are needed to assess the effect of this difference of seed coats sculpturing in order to find out more on their functional roles.

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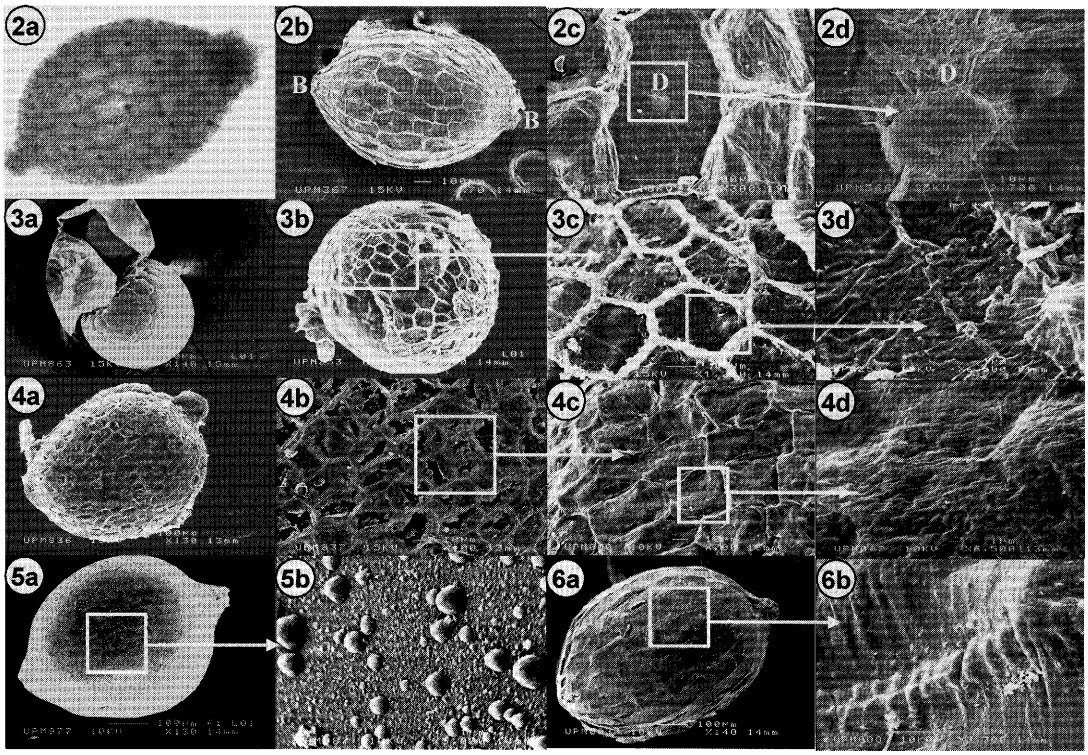

Fig. 2. a) Sub-globose seed of *Halophila beccarii*. The seed coat has reticulation with a pale brown peg in the center of the reticulation (observed under dissecting microscope, Mag: 0.63X; b) SEM micrographs showing the blunt ends (B) of the seed and the seed coat with distinct reticular patterns and a dot in the center of each reticulation; c) Rectangular reticulation and dot (D), scale bar=100 μm; d) An enlarged dot (D) which is an irregular short peg in the center of the reticulation and the surrounding surface with microsculptures, scale bar=10 μm.

Fig. 3. a–b) SEM micrographs showing the seed coat surface of *Halophila decipiens* with hexagonal reticulation, scale bar=100 μm, 10 μm; c-d) The surface within the reticulation is covered with microsculptures, scale bar=10 μm, 1 μm.

Fig. 4. a) Seed of *Halophila ovalis* has a distinct reticulation; b-c) Showing the isometric reticulation, scale bar=10 μm; d) showing the microsculptures covering the surface within the reticulation, scale bar=1 μm.

Fig. 5. a) SEM micrographs showing the seed coat of *Halophila* sp. with no reticulation, scale bar=100 μm; b) An enlarged seed coat surface view, verrucose in appearance sculptured with numerous small blunt projections, scale bar=1 μm. Cf. *H. spinulosa* seed coat which is characteristically sculptured with numerous long small peg-like projections (Birch 1981).

Fig. 6. a) The globular or sub-spherical seed of *Halophila stipulacea* is usually with blunt ends and with no distinct reticulation, scale bar=100 μm; b) The boundaries of the reticulation has a peculiar fish bones suture, scale bar=10 μm.

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