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Research article

Reproductive organ development of tropical seagrass, *Enhalus acoroides*

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Abstract

The reproductive organ development and anatomical structures of *Enhalus acoroides* using samples collected from Haad Chao Mai National Park on the Andaman Sea, southern Thailand, were examined. Details were investigated of the different developmental stages of four different reproductive organs: male inflorescence and female inflorescence with a solitary flower, fruit and seed. A newly reported observation was that the similarity in size and shape between male and female papillae facilitated the hydrophobic pollination process. The papillae of the male floret and female flower were newly reported. The mean values (Mean ± SD) of the papillae of male florets were 31.5 ± 3.1 µm long and 25.0 ± 3.6 µm wide and those of female flowers were 31.0 ± 2.9 µm long and 26.5 ± 1.9 µm wide. The development of fibers around the female peduncle was newly reported. After the seed attached to the sediment, the epidermal cells at the base of hypocotyl modified to various long filaments in order to act like root hairs within 7 d. The results indicated that the characteristics of reproductive organs of *E. acoroides* are linked to the reproductive biology, which substantially improves understanding of the biological adaptation for promoting sexual reproduction of this seagrass.

Introduction

Seagrasses are flowering plants which grow fully submerged and rooted in shallow marine and estuarine environments worldwide (Spalding et al., 2003). The distribution of seagrass depends on several factors including temperature, precipitation and wind strength (Phillips and Meñez, 1988). Seagrasses grow in areas dominated by soft substrates such as sand or mud, but some species can be found growing on rocky substrates (Spalding et al., 2003). Among tropical seagrasses, *Enhalus acoroides* is the largest and provides a variety of valuable ecological benefits to coastal areas; the species is widely distributed in the Indian and Pacific Oceans, and the Red Sea (Green and Short, 2003). The leaves and detritus of this species are a source of food for sea turtles, fish, waterfowl and, especially, dugong (Adulyanukosol and Poovachiranon, 2003; Adulyanukosol et al., 2010).

Pollination is a key process in the life cycle of flowering plants. It involves a vector responsible for the transport which can be either biotic (animals) or abiotic (usually wind). Another abiotic vector is water-flow, which is named hydrophilic pollination, with most

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seagrass species utilizing hydrophilic pollination (Van Tussenbroek et al., 2016). *Enhalus acoroides* is the only seagrass species that shows hydrophobic pollination (den Hartog, 1970; Phillips and Meñez, 1988). The pollination periods of *Enhalus acoroides* have hitherto been observed and reported at low tide, a few days after a new moon and after a full moon (den Hartog, 1970; Brouns and Heijs, 1986). This pollination feature restricts the distribution to a region from the shallow water above the lowest low water to a depth of 5 m (Supanwanid and Lewmanomont, 2003). The flowering and successful fruit formation occurs year round (den Hartog, 1970; Brouns and Heijs, 1986; Kiswara, 1996; Ogawa and Nanba, 2002; Rollón et al., 2003). The young fruit appears 1 mth after the observation of female flowers (Brouns and Heijs, 1986). Although the morphology of *E. acoroides* flowers and fruit has been reported extensively (Troll, 1931; den Hartog, 1970; Phillip and Meñez, 1988; Kuo and den Hartog, 2001), their anatomy and development are not adequately understood. This study provided details of the development of the reproductive organs of *E. acoroides* through its morphological and anatomical structures. Some characteristic of flower papillae, peduncle and hypocotyl were newly reported. These morphological characters may have adaptive significance and be related to its sexual reproduction.

Materials and Methods

Collection of plant materials

Samples of *Enhalus acoroides* (L.f.) Royle were collected from the shoreline at Haad Chao Mai National Park, Trang Province, southern Thailand. The collection site was at 7° 23.4' N and from 99° 20.16' E to 99° 19.64' E. Samples of male inflorescence, female inflorescence and fruits at different stages of development were selected and preserved for study.

Morphological studies

Fresh plants were selected and classified into male and female plants. Prior to observation under a stereomicroscope, the leaf sheaths from the rhizome were peeled off to identify the developmental stage of the reproductive organs.

Anatomical studies

For anatomical studies, the selected specimens were fixed in a weak chromium acetic acid solution for 24 hr at room temperature (Johansen, 1940). Permanent slides were made using a paraffin method (Johansen, 1940; Kermanee, 2008). Serial sections of 10–15 µm were cut in transverse and longitudinal planes and stained with 1% Safranin and 0.5% Fast Green prior to mounting with permount solution.

Medium preparation and seed culturing

In total, 20 seeds were used in the current study. The seeds of *E. acoroides* were removed from the sterilized fruits. These seeds were then transferred into artificial seawater media (Aquaraise Reef Salt). The medium was adjusted to pH 5.8, using 1 N HCl or 1 N NaOH, prior to autoclaving (121°C for 15 min under 1.034 bar). All cultures were kept at 25±2°C under a light intensity of 40 µmol/m^2/s and a 16 hr daylight photoperiod provided using white fluorescent lamps and maintained in a clean culture room. Any morphological or anatomical developments of seedlings were examined for 7 d.

Results and Discussion

Male inflorescence

The male inflorescence development was observed from the early stage to maturation. Four samples of young inflorescence at different stages could be found unilaterally at a vertical rhizome of a fertile male plant (Fig. 1A). Stage 1, the youngest inflorescence bud, occurred in a specific position at a shoot apical meristem beside its leaf primordium (Fig. 1A). The elder floral bud was between the second and the fourth leaves. Stage 2, during male flower development, the inflorescence bud produced a peduncle and a dome shaped reproductive apex in between the spathe primordium. At this stage, the flower primordium was clearly different from stage 1 and had started to grow larger. Stage 3 began when the floret primordia were visible. Spathes covered the initial axis and the florets were generated afterward. The peduncle became longer and the epidermis at the tip of the outer spathe was modified to become the initial trichomes (Figs. 1B–C).

During stage 4, the elongation of the peduncle continued concurrently with an increase in the size of the developing trichome primordium to 300–500 µm. Two overlapping hyaline spathes with long trichomes were clearly observed. The outer spathe developed subsequently to cover the inner spathe and florets inside. The terminal florets were more developed than the ones below, showing cymose inflorescence type (Figs. 1D–E).

In stage 5, the long trichomes became visible. Trichomes on each spathe developed into five rows and became longer, especially in the middle row (Fig. 2A). Primordia of the florets were clearly detected and showed well-grown terminal florets (Fig. 2B).

In stage 6, the petal and sepal primordia became visible. The pedicel became longer, making the florets separate from each other. The peduncle was whitish at 4.0–10.0 cm long and the inflorescence became larger (Figs. 2C–D).

Stage 7 began when the stamens became visible at their base and the florets loosened because of the well-developed pedicel (Figs. 2E–F). Each inflorescence contained 636.5 ± 75.3 (n = 30) actinomorphic florets. The sepals grew to completely cover the floral bud (Fig. 2G).

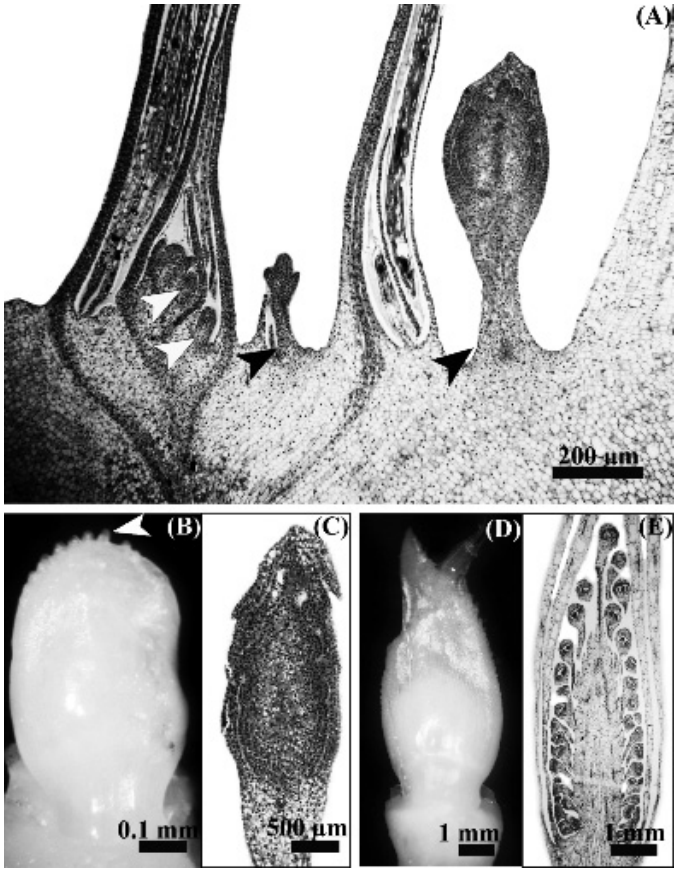

Fig. 1 Stages 1 to 4 of developments of *Enhalus acoroides* male inflorescence: (A) longitudinal section of shoot showing four young inflorescence in three different stages, inflorescence buds (white arrows) and stage 2 and 3 of male inflorescences (black arrows); (B–C) stage 3, (B) epidermis at tip of the outer spathe (arrow), (C) origin of numerous florets; (D–E) stage 4, (D) two overlapping spathes with trichomes, (E) longitudinal section showing cymose inflorescence type

In stage 8, each spathe was broadly lanceolate, 4.0–6.0 cm long and 2.0–4.0 cm wide, slightly curled at the margin with an obtuse apex. The peduncle became light green. During blooming, spathes became cirrhous and opened to release florets to the water (Fig. 3A). Each anther produced numerous pollen grains. When the florets were released, the sepals and petals flip over their pedicel and three white stamens opened and became exposed to the air (Figs. 3B–D). Each floret had a thin whitish pedicel, 5.0–15.0 mm in length. The male floret was whitish, 2.0–3.0 mm long and 0.7–1.2 mm in diameter. The floret consisted of three contorted white sepals and three valvate white petals. The sepal was 1.5–2.0 mm long and 0.5–1.0 mm wide. The sepal consisted of a single layer of epidermis on both the adaxial and abaxial surfaces. At the centre of sepal, numerous secretory cells were found in between two layers of epidermis. Each petal was ovate, 1.5–1.8 mm long and 0.5–1.2 mm wide. The epidermal cells of the petals were modified to papillae, 31.5 ± 3.1 µm long (n = 50) and 25.0 ± 3.6 µm wide (n = 50). The angle between papillae was 67.4 ± 20.1 degrees (n = 50). Papillae were observed on the adaxial surface of petals and on the anther wall (Figs. 4A–C). The stamen was 1.0–1.5 mm long with a basifixed filament. Each anther consisted of four pollen sacs carrying numerous pollen grains. Mature pollen grain was monadal and spherical, 70-150 µm in diameter, without aperture or inaperturate. The pollen wall structure consisted of two layers called exine and intine (Figs. 4D–E).

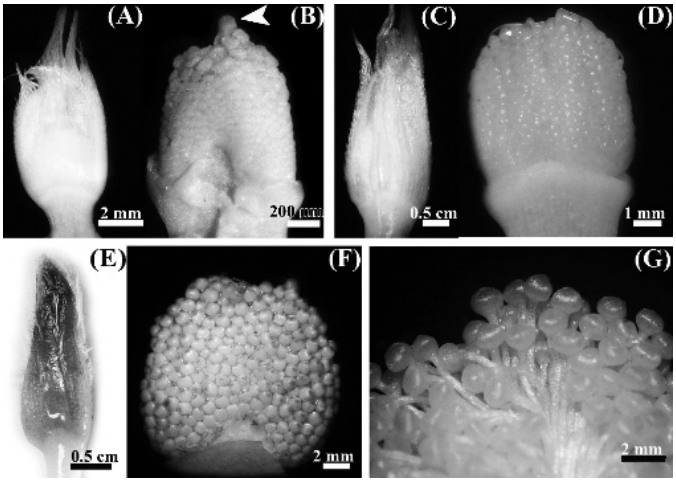

Fig. 2 Stages 5 to 7 of developments of *Enhalus acoroides* male inflorescence: (A–B) stage 5, (A) outer spathe with five rows of trichomes, (B) inflorescence showing well grown terminal floret (arrow); (C–D) stage 6, (C) two overlapping spathes with long trichomes, (D) florets on the axis; (E–G) stage 7, (E) two overlapping spathes covering male florets, (F) numerous florets on axis, (G) florets with long pedicels

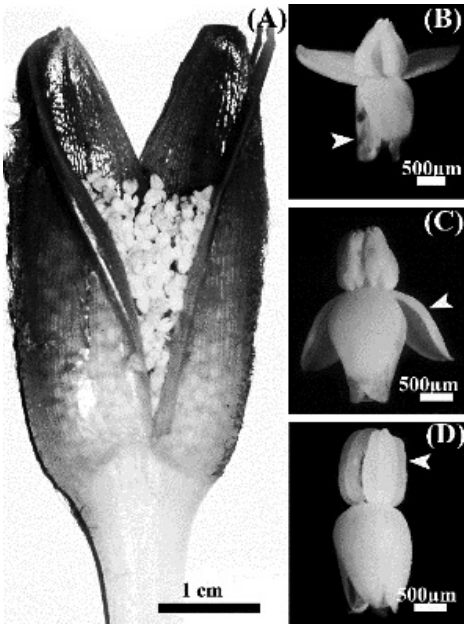

Fig. 3 Stage 8 of *Enhalus acoroides* male inflorescence: (A) inflorescence showing cirrhous spathes and numerous florets; (B–D) blooming of floret, (B) sepals (arrow) flipped over and covering their pedicel, (C) petals (arrow) flipped over sepals, (D) completely bloomed floret showing disclosed anther (arrow)

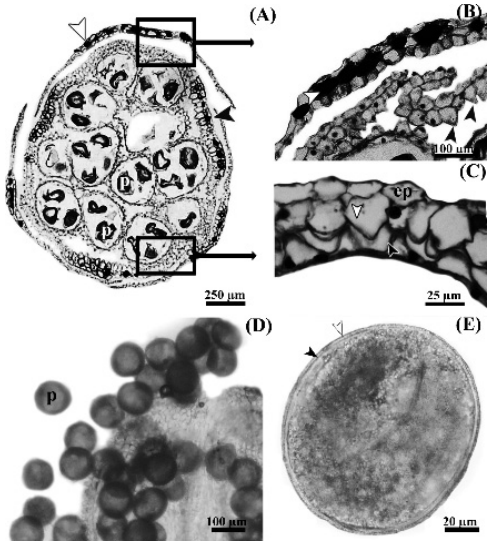

Fig. 4 Anatomy of *Enhalus acoroides* male floret: (A): cross section of floret showing anthers with pollen (p), sepal (white arrow) and petal (black arrow); (B) cross section of sepal and petal showing papillae (black arrow) on adaxial surface of petal and secretory cells of sepal (white arrow); (C) cross section of petal and anther wall, showing papillae on adaxial surface of petal (white arrow), epidermis, and papillae on anther wall (black arrow); (D) mature pollen grains; (E) mature pollen grain showing outer layer, exine (white arrow), and inner layer, intine (black arrow), where ep = epidermis; p = pollen grain

Female inflorescence (with solitary flower)

Four different stages of immature female flowers were found unilaterally in a single shoot. Stage 1, the first sign of flower primordium formation, occurred between the leaf primordia (Figs. 5A–B). In stage 2, the inflorescence bud developed its outer spathe to cover the initial axis. In this stage, the inner spathe primordia were visible. The peduncle became longer (Fig. 5C). Stage 3 began when the sepal primordia became visible and the peduncle became longer. The inner spathes grew to completely cover the primordia of sepals (Fig. 5D). Stage 4, began when the sepal primordia elongated. The sepals grew to completely cover the petal primordia. The inflorescence bud developed its outer spathe, inner spathe, sepals, petals, and ovary, respectively (Fig. 5E).

Stage 5 commenced after the outer spathe completely covered the inner spathe and the epidermis at the tip of the outer spathe modified to become initial trichomes (Fig. 6A). Sepals continued to lengthen relatively rapidly. The pistil was composed of a short inferior ovary (Fig. 6B). In stage 6, the long trichomes became visible. Trichomes on each spathe developed into five rows and elongated, 1.54 ± 0.63 mm in length (Fig. 6C). The wall of the inferior ovary became longer and the ovary was clearly observed (Fig. 6D). The inferior ovary was 2.0 mm in diameter and 2.5 mm in length. In stage 7, trichomes on each spathe developed into three rows and became longer, especially the middle row, 6.10 ± 2.18 mm in length (Fig. 6E). Sepals completely covered petals and the stigma initial tissue. The epidermis of the wall of inferior ovary was modified to initial trichomes (Fig. 6F).

In stage 8, the spathes became longer with numerous trichomes, which developed into five rows. The trichomes lengthened to 8.88 ± 2.55 mm (Fig. 6G). During this stage, the trichome primordia of the inferior ovary wall elongated to 4.68 ± 1.76 mm in length. The ovary, 20 mm in length, was much longer than in the previous stage. The ovule was initiated (Fig. 6H).

In stage 9, the completed female inflorescence composition was visible. The spathes, sepals, petals and pistil including the ovule had completely developed at this stage (Figs. 6I–J).

In stage 10, the mature spathe was obtuse, 4–6 cm long and 1–3 cm wide. Five rows of trichomes, 9.74 ± 2.12 mm in length, occurred along each spathe. The wall of the inferior ovary had dense trichomes (7.14 ± 1.79 mm in length) near the base of sepal. The tip of the spathe stretched out showing the solitary female flower (Figs. 6K–L). The female flower was radial symmetric and had a long inferior ovary, 25 mm in length (Fig. 7). The ovule-bearing part was a syncarpous gynoecium, consisting of hexagynous fused carpels (Fig. 7C). The ovary was composed of six locules and each locule contained 1–3 anatropous ovules. The ovary was rippled and covered with short trichomes (Fig. 7D).

In stage 11, the sepals and petals bloomed. There were three petals and three sepals. After blooming, the creased petals expanded on the water surface (Fig. 8A). The valvate petal was whitish and oblong, 30–50 mm long and 20–30 mm wide. The petal consisted of one layer of epidermis on both the abaxial and adaxial surfaces. The epidermal cells were modified to papillae, especially the adaxial epidermis (Fig. 8B). Each papilla was 31.1 ± 2.9 µm long (n = 50) and 26.5 ± 1.9 µm wide (n = 50). The angle between papillae was 61.5±19.2 degrees (n = 50). The contorted sepal was white-reddish and oblong, 10–15 mm long and 5–7 mm wide, with a rounded apex and reticulate margin. The sepal consisted of one layer of epidermis on both the adaxial and abaxial surfaces, and a layer of parenchymatous mesophyll (Fig. 8C).

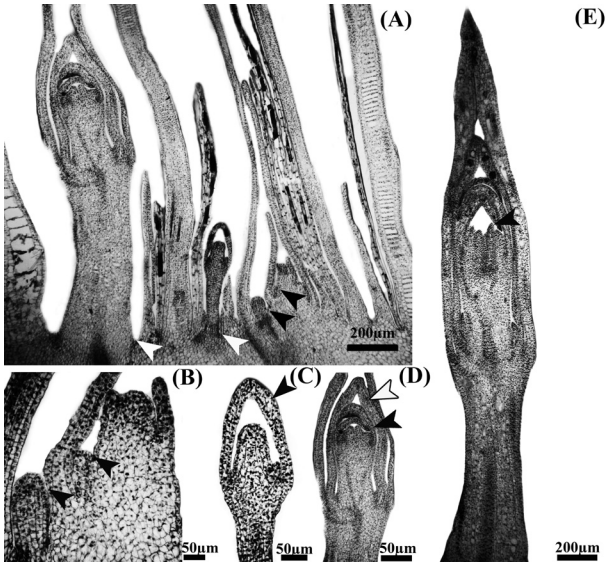

Fig. 5 Stages 1 to 4 of developments of *Enhalus acoroides* female inflorescence: (A) shoot with different growth stages of female inflorescence, stage 1 (black arrows), and stages 2 and 3 (white arrows); (B) stage 1 of female inflorescence buds (arrows); (C) stage 2 showing spathe formation (arrow); (D) stage 3 showing sepals (black arrow) covered by spathes (white arrow); (E) stage 4 showing petal formation (arrow)

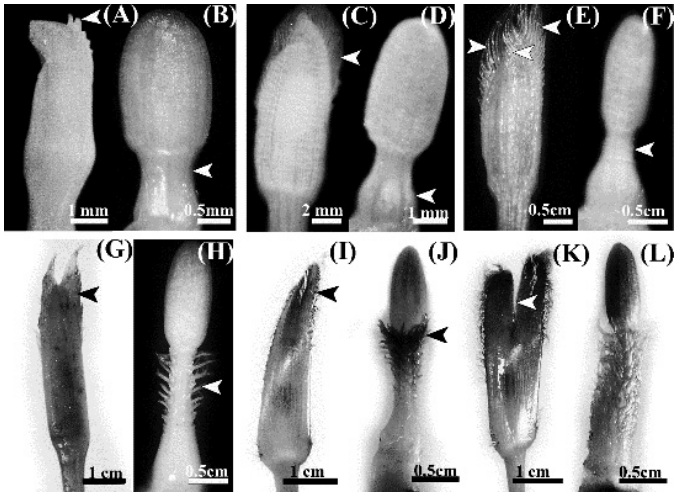

Fig. 6 Stages 5 to 10 of developments of *Enhalus acoroides* female inflorescence: (A–B) stage 5, (A) spathe with initial trichomes (arrow), (B) female flower with short inferior ovary (arrow); (C–D) stage 6, (C) spathe with short trichome (arrow), (D: female flower showing ovary (arrow); (E–F) stage 7, (E) spathe with three rows of trichomes (arrows), (F) initial trichomes on wall of inferior ovary (arrow); (G–H) stage 8, (G) spathe with numerous trichomes (arrow), (H) female flower with trichomes on wall of inferior ovary (arrow); (I–J) stage 9, (I) spathe with five rows of trichomes (arrow), (J) female lower showing numerous trichome near base of sepal (arrow); (K–L) stage 10, (K) mature flower showing detached spathes (arrow), (L) solitary mature female flower

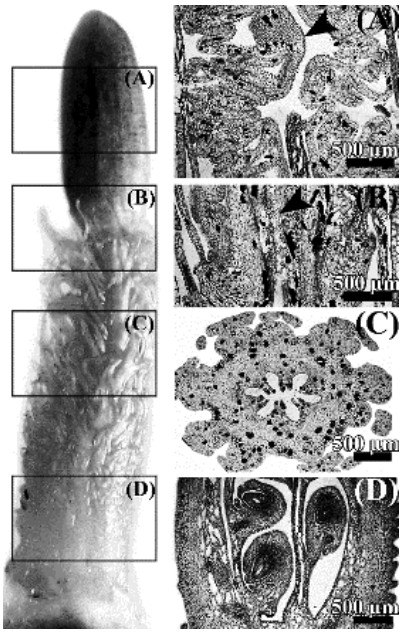

Fig. 7 Anatomical structures of *Enhalus acoroides* female flower (stage 10): (A) longitudinal section showing sepals and creased petals (arrow); (B) longitudinal section of stigma showing numerous papillae (arrow); (C) cross section of pistil showing stylar canals; (D) longitudinal section of ovary showing ovary wall and number of ovules per locule

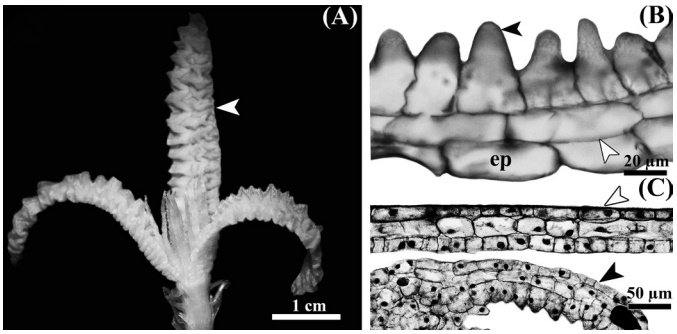

Fig. 8 Stage 11 of *Enhalus acoroides* female flower: (A) creased petal (arrow); (B) cross section of petal showing epidermis, mesophyll (white arrow) and papillae on adaxial surface (black arrow); (C) longitudinal section through sepal (white arrow) and creased petal (black arrow) showing anatomical structure, where ep = epidermis

The peduncle of the female inflorescence is very important for hydrophobic pollination and fruit production. The development of the peduncle from early immature, late immature, before pollination, and after pollination stages were observed. In the early immature peduncle, two vascular bundles originated at the center and were surrounded by parenchyma cells without air lacuna (Fig. 9A). The secretory cell and fiber were not evident in this stage. During the late immature stage, small vascular bundles were produced around the two large vascular bundles. Parenchyma cells differentiated to aerenchyma cells, producing numerous air lacunae around two large bundles (Fig. 9B).

In the mature stage before pollination, the female peduncle was 30–60 cm long. The air lacunae became larger and occupied most of the space in this organ. Two large vascular bundles remained at the center and were encircled by 18–20 small bundles and air lacunae. Secretory cells were scattered from the epidermis to the center. The fiber developed mostly in the outer area of the small vascular bundles (Figs. 9C–E).

After pollination when the fruit became larger, the peduncle coiled and contracted around the shoot near the base. The fibers were more developed on the outer side of the coil than the inner side. On the outer side of each coil, the fiber were mostly only in the outer area of bundles. On the inner side of a coil, the fiber developed much better on the same side of the coil, that is the inner area of bundles; as a result, the bundles were enclosed by fiber (Figs. 9F). In addition, the hypodermal cells on the outer side (111.42 ± 9.13 µm, $n = 20$) of a coil were substantially longer than the cells on the inner side of the coil (70.56 ± 12.09 µm, $n = 20$) (Figs. 9G, 9H).

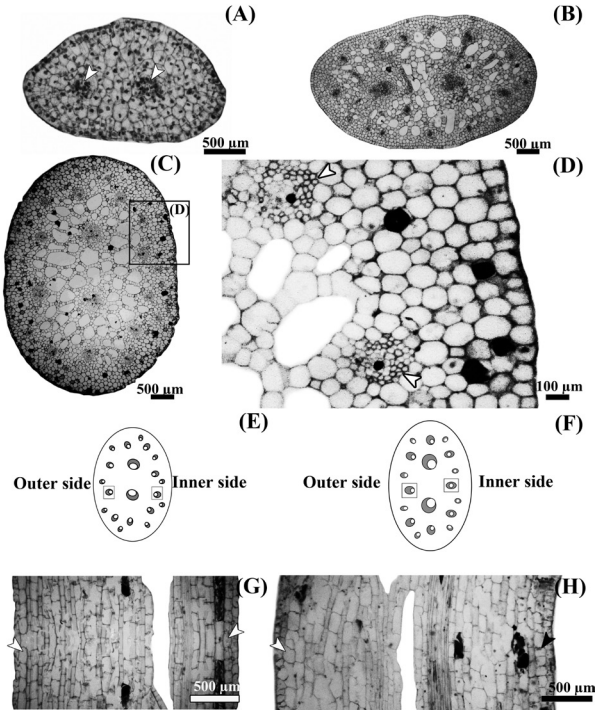

Fig. 9 Female peduncle of *Enhalus acoroides*: (A) immature peduncle showing two vascular bundles at the center (arrow); (B) two large bundles and small vascular bundles; (C) mature peduncle showing two large vascular bundles are encircled by 18–20 small bundles and air lacunae; (D) fibers in outer region of vascular bundles (arrows); (E) position of fiber of uncoiled peduncle; (F) position of fiber of coiled peduncle; (G) longitudinal sections of uncoiled peduncle showing hypodermal cell (white arrows); (H) longitudinal sections of the coiled peduncle showing the hypodermal cell at the outer side (white arrow) and the inner side (black arrow) of the coil

Fruit

During the immature stage of fruit formation, seeds were not completely developed. The fruit was covered by two overlapping spathes (Fig. 10A). When the fruit developed and became larger, the spathes opened wide and the persistent style was observed as a long beak, 0.5–1.0 cm in length. The outer layer of pericarp was covered with dense trichomes during the immature stage (Fig. 10B). The mature fruit was oblate to ovoid, 4–7 cm long and 3–5 cm in diameter (Fig. 10C). The apex of mature fruit became larger. Two persistent spathes were 4-6 cm long and 1–3 cm wide, covered by 6–8 longitudinal rows of trichomes. The pericarp was 5–10 mm thick. The mesocarp was composed of vascular bundles, ground parenchyma and numerous secretory cells (Fig. 10C). In the late mature stage, the ripened fruit dehisced lengthwise along the smooth pericarp and released 11.63 ± 5.51 angular seeds (Figs. 10D–10E).

Seed

Enhalus acoroides produces seeds that germinate before detaching from the fruit. The seedling germinates and grows by using its own energy while still attached to the mother plant, called viviparous seed. Seed development was observed from the early stage to maturation. In the early stage, the seed coat developed from the integuments of the ovule. The outer integument developed into testa and the inner integument developed to tegmen (Figs. 11A–B). During the immature stage, the seed coat was well developed and composed of tightened parenchymatous cells in many layers, especially near the top of the seed (Figs. 11C–11D). When the seed matured, seed coat was 100–450 µm wide (Figs. 11E–11F). The seed coat near the top breaks down to form large air lacunae. When the fruit had naturally ripened and dehisced, seed the coat immediately autolyzed.

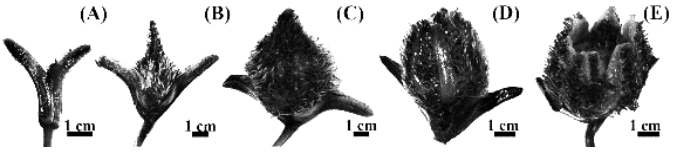

Fig. 10 Fruit development of *Enhalus acoroides*: (A) Immature fruit showing two overlapping spathes; (B) long beak of immature fruit; (C) fleshy fruit with two spathes; (D) mature fruit showing two persistent spathes; (E) ripened fruit

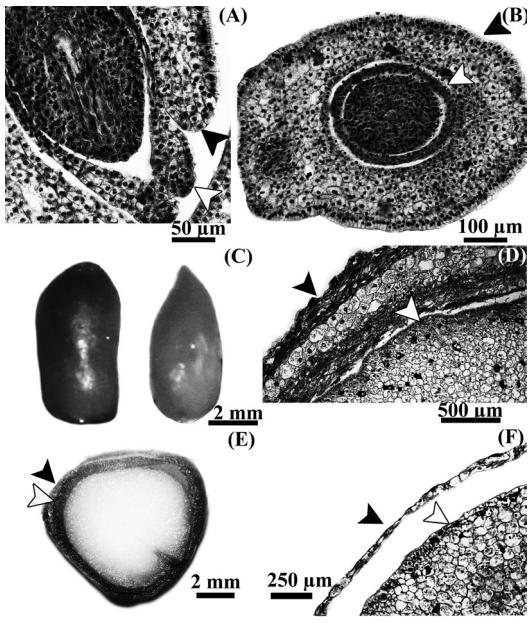

Fig. 11 Seed coat of *Enhalus acoroides*: (A) longitudinal section of ovule showing outer (black arrow) and inner (white arrow) integument; (B) cross section of ovule showing outer (black arrow) and inner (white arrow) integuments; (C) immature seed with seed coat (left) and without seed coat (right); (D) cross section of seed showing immature seed coat (black arrow) and seed (white arrow); (E) cross section of mature seed showing thin membranous seed coat (black arrow) and hypocotyl (white arrow); (F) cross section of mature seed showing thin membranous seed coat (black arrow), and seed (white arrow)

The mature seed was 1.0–2.0 cm long, 1.0–1.5 cm in diameter, and its weight was 1.12 ± 0.05 g (n = 15). The mature seed had an embryo with a distinct leaf primordium, protected by a cotyledon and an enlarged hypocotyl. The shoot apical meristem was dome shaped and consists of two-layered tunica. Cell division occurred on the anticlinal plane or perpendicular to surface. The corpus was the cell in the central region of the apical meristem. Cell divisions occurred in the anticlinal and periclinal planes (Fig. 12B). The mature seed had a provascular bundle extending from the base of the embryo to the base of hypocotyls and the apex (Fig. 12A). Secretory cells and abundant starch grains were found at the base of hypocotyl tissue (Fig. 12C). After the seed detached, it dropped into the sediment beside the mother plant. The wall of the epidermal cells at the base of hypocotyl became thicker and modified to various long filaments (within 7 d) acting like root hair (Fig. 13).

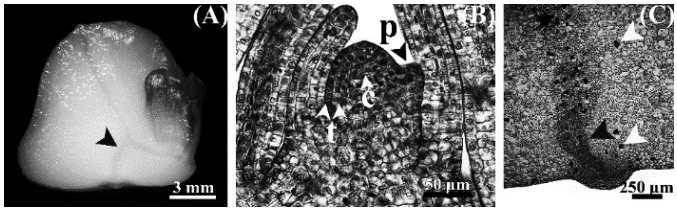

Fig. 12 Mature seed of *Enhalus acoroides*: (A) longitudinal sections of mature seed through the embryo showing provascular bundle (arrow); (B) longitudinal section of seed through the embryo showing apical meristem with distinct leaf primordium (p), two-layered tunica (t) and corpus (c); (C) longitudinal sections of mature seed through base of hypocotyl showing large starch-rich hypocotyls, secretory cells (white arrow) and provascular bundle (black arrow) at the base portion, where c = corpus; p = leaf primordiuim; t = tunica

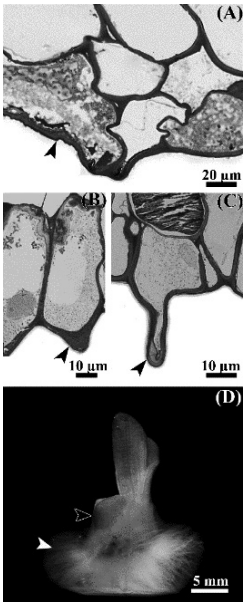

Fig. 13 Seedling of *Enhalus acoroides*: (A) thick wall of epidermal cell (black arrow) after 2 d; (B) filament origin (black arrow) on the outer tangential side after 4 d; (C) slender filament (black arrow) after 6 d; (D) seedling after 8 d showing numerous slender filaments (white arrow) and hypocotyl (black arrow)

The current morphological and anatomical data provided insight into the development of *Enhalus acoroides* reproductive organs from the early to mature stages. Table 1 provides a comparison of some of the morphological characteristics of *E. acoroides* reported in the current study with other previous studies (Kaul, 1968; den Hartog, 1970; Brouns and Heijs, 1986; Phillip and Meñez, 1988; Kuo and McComb, 1989; Rollón, 1998; Kuo and den Hartog, 2001; Vermaat et al., 2004; Kuo and den Hartog, 2006). In addition, the current study is the first to report the detailed morphological features of papillae, which are related to the pollination mechanism.

In the early stages of flower production, male or female inflorescence buds were produced unilaterally at axillary of leaf primordia. When the inflorescence detached, an inflorescence scar developed as reported by Rollón (1998); in the current study, the inflorescence scar was positioned close to every other leaf scar or node.

Enhalus acoroides produces male inflorescence all year round to release male florets to increase the chances of pollination. den Hartog (1970) reported that the petals of male florets had a waxy cuticle and thus were hydrophobic. However, the current morphological and anatomical studies indicated that hydrophobic interaction may be caused by not only the waxy cuticle but also by the papillae and the nastic movement mechanism. The male florets drifted just after release from the inflorescence and the sepals spectacularly flipped over on the water surface. During low tide, when the female flower reached the water surface, petals of mature female flowers consisting of numerous papillae on the adaxial surface were exposed on the water surface. The creased petals of female flowers expanded and waited for male florets. Female flowers and male florets easily attached and locked to each other by their numerous papillae which have similar shapes and sizes. As the water level rose with the incoming tide, female petals engulfed the male florets. The anthers shed pollen grains that were discharged onto the stigmatic papillae. Because the pollen grain is inaperturate, the pollen tube can emerge from any pollen region. Consequently, pollination occurs underwater, as den Hartog (1971) reported that pollination cannot take place on the water surface, because the styles are covered by the perianth leaves and the anthers rise above the water level. Tanaka et al. (2004) reported that the pollen wall of *E. acoroides* was reticulate and had a discontinuous exine structure on the intine, which correlated with underwater pollination and the aerial pollination mechanism.

After pollination, the peduncle became coiled and contracted around the shoot near its base. Anatomical studies showed that the vascular bundle on the inner side of the coiling peduncle were surrounded by numerous fibers. This characteristic role can be found in the coiling and twining organs of vines, and gelatinous fibers occur concomitantly with the ability to coil (Bowling and Vaughn, 2009).

Table 1 Comparative analysis of reproductive morphological characteristics of *Enhalus acoroides*

Character	Current study	Reference								
		1	2	3	4	5	6	7	8	9
Locality	Thailand	Singapore	ns	Papua New Guinea	ns	Australia	Philippines	ns	Philippines	ns
Male flower										
Type	Cymose	*	Cymose	*	*	*	*	*	*	*
Peduncle color	Whitish	*	*	*	*	*	*	*	*	*
Peduncle length (cm)	4–10	*	5–10	*	*	Short	*	5–10	*	*
Spathe (number/ shape)	2/ Lanceolate	*	2/Lanceolate	*	*	*	*	*	*	*
Spathe length/ width (cm)	4–6/ 2–4	*	5–10/ 3	*	*	*	*	*	*	*
- Row of trichome	5	*	*	*	*	*	*	*	*	*
Floret symmetry	Radial	*	*	*	*	*	*	*	*	*
- Number of florets	636.5±75.3 (n=30)	*	*	*	*	*	531±39 (n=3)	*	400–500	*
Pedicel color	Whitish	*	*	*	*	*	*	*	*	*
Pedicel length (mm)	5–15	*	3–12	*	*	*	*	3–12	*	*
Number of sepals	3	*	3	*	*	Tepals=6	*	3	*	Tapals=6
- Sepal color	White	*	White	*	White	*	*	White	*	*
- Sepal aestivation	Contorted	*	*	*	*	*	*	*	*	*
- Sepal length/ width (mm)	1.5–2.0/ 0.5–1.0	*	2/ *	*	2/ *	*	*	2/*	*	*
Number of petals	3	*	3	*	*	*	*	3	*	*
- Petal color	White	*	White	*	White	*	*	White	*	*
- Petal aestivation	Valvate	*	*	*	*	*	*	*	*	*
- Petal length/ width (mm)	1.5–1.8/ 0.5–1.2	*	*	*	1.75/ *	*	*	1.75/*	*	*
- Papillae length (μm)	31.5±3.1 (n=50)	*	*	*	*	*	*	*	*	*
- Papillae width (μm)	25.0±3.6 (n=50)	*	*	*	*	*	*	*	*	*
Number of stamens	3	*	3	*	*	3	*	3	*	3
- Anther color	White	*	*	*	White	*	*	White	*	*
- Anther length (mm)	1.0–1.5	*	1.5–1.7	*	*	*	*	1.5–1.7	*	*
- Fixation of filament	Basifixed	*	Subsessile	*	*	*	*	*	*	*
Pollen grain size (μm)	70–150	*	170–175	*	*	*	*	175	*	*
Female flower										
Type	Epigynous	*	*	*	*	*	*	*	*	*
Flower symmetry	Radial	*	*	*	*	*	*	*	*	*
Peduncle length (cm)	30–60	*	40–50	*	40-50	*	30–150	40–50	*	*
Spathe (number/ shape)	2/ obtuse	*	2/ obtuse	*	*	2/ *	*	*	*	2/ *
Spathe length/ width (cm)	4–6/ 1–3	*	4–6/ 1–2	*	*	*	*	*	*	*
Number of sepals	3	3	3	*	*	3	*	3	*	3
- Sepal color	Reddish	Green	Reddish	*	Reddish	*	*	*	*	*
- Sepal aestivation	Contorted	*	*	*	*	*	*	*	*	*
- Sepal length/ width (mm)	10–15/ 5–7	*	*	*	*	*	*	*	*	*
Number of petals	3	3	3	*	*	3	*	3	*	3
- Petal color	Whitish	White	White	*	White	*	*	White	*	*
- Petal aestivation	Valvate	*	*	*	*	*	*	*	*	*
- Petal length/ width (mm)	30–50/ 20–30	*	40–50/ 3–4	*	40–50/3–4	*	*	*	*	*
- Papillae length (μm)	31.0±2.9 (n=30)	*	*	*	*	*	*	*	*	*
- Papillae width (μm)	26.5±1.9 (n=30)	*	*	*	*	*	*	*	*	*
Pistil	Syncarpous	*	*	*	*	*	*	*	*	*
- Stigma branch	2	2	*	*	*	*	*	*	*	*
- Style character	6 fused	6 fused	6	*	*	*	*	6	*	6
- Ovary position	Inferior	*	*	*	*	*	*	*	*	*
- Ovules type	Anatropous	*	*	*	*	*	*	*	*	Anatropous
- Number of ovules	15.47±5.11 (n=30)	*	Several	*	*	*	*	*	*	*
Fruit										
Fruit shape	Oblate to ovoid	*	Ovoid	*	*	*	Ovoid	Ovoid	*	*
Fruit length/ width (cm)	4–7/ 3–5	*	5–7/ *	*	5–7/ *	6/ *	5–7/ *	5–7/ *	*	6/ *
Pericarp thickness (mm)	5–10	*	*	*	*	*	*	*	*	*
Beak of fruit (mm)	5–10	*	*	*	*	*	*	*	*	*
Persistent spathe (length/ width) (cm)	4–6/ 1–3	*	*	*	*	*	*	*	*	*
Seed										
Seed number within a fruit	6–20,11.63±5.51 (n=30)	*	8–14	2–11	*	*	3–15	*	5–13	2–6
Seed weight (g)	1.12±0.05 (n=15)	*	*	*	*	*	*	*	*	*
Seed coat thickness (μm)	100–450	*	*	*	*	*	*	*	*	*
Seed shape	Angular	*	Obconical	*	*	Angular	Obconical	*	*	*
Seed length/ width (cm)	1.0–2.0/ 1.0–1.5	*	1.0–1.5/ *	*	1.0–1.5/ *	*	1.0–1.5/ *	1.0–1.5/*	*	*

* = not reported, ns=not specific

References cited: 1=Kaul (1968), 2=den Hartog (1970), 3=Brouns and Heijs (1986),
4=Phillip and Meñez (1988), 5=Kuo and McComb (1989), 6=Rollón (1998), 7=Kuo and den Hartog (2001), 8=Vermaat et al. (2004), 9=Kuo and den Hartog (2006)

The viviparous seed of *Enhalus acoroides* has an embryo with a distinct leaf primordium which is protected by enlarged hypocotyls. The starch in the seed base is stored for use during embryo development. The hypocotyl not protects the developing embryo but also acts as a source of nutrition for the seedling (Montaño et al., 1999). Nutrient storage in the form of starch grains has been reported in another seagrass species, *Thalassia*, for seed germination (Kuo et al., 1991). The seeds of seagrass also contain other nutrients such as vitamins, protein, carbohydrates, polyunsaturated fatty acids and antioxidants (Montaño et al., 1999).

Kuo and den Hartog (2001) reported that the angular seeds of *Enhalus acoroides* were covered by a thin membrane. In the current study, the apical portion of seed was covered by a white seed coat consisting of parenchyma cells and large air lacunae. When the fruit ripened and its pericarp broke off, the water motion in the sea wafts and places the seed near the mother plant. The soft and thin seed coat may not support the nutrient rich seed to drift far from the mother plant because of autolysis. This may not be consistent with Lacap et al. (2002), who reported that the seeds of *E. acoroides* floated at a speed of 0.26 ± 0.02 km/hr and seed flotation times were up to 30 min. In their experiment, the seeds were spread out over the water surface of a flow-through sea water tank. However, the *E. acoroides* seeds naturally detached from ripened fruit near the sediment and dropped near its mother plant. However, the early mature seed might float as Lacap et al. (2002) reported while it is still covered by the seed coat composed of aerenchymatous tissue. The dome shape of the seed and starch grain richness at the hypocotyl base allow the seed to stand on the mud in the right position before the first root occurs. In addition, the air lacunae with less starch grain occupy the top portion of seed and probably store other substances (Kuo et al., 1991). After the seed reached the sediment in the current study, it produced numerous long filaments, which may support the seedling and absorb nutrients. It was apparent that *E. acoroides* has developed its reproductive organ characteristics in many ways to promote the possibility of pollination and to increase the chance of surviving and broadening its distribution.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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