

Effects of pH on growth and anatomical characters of tape seagrass (*Enhalus acoroides* (Linnaeus f.) Royle)

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Received 1 Dec 2024, Accepted 28 Jul 2025

Available online 18 Aug 2025

ABSTRACT: *Enhalus acoroides* (Linnaeus f.) Royle or tape seagrass plays a vital role in tropical seagrass meadows, especially in Thailand. While ocean acidification negatively affects many marine species, it may benefit tropical seagrasses. *E. acoroides* relies on seed dispersal for sexual reproduction, and pH variations may influence seedling development. This study examined the effects of pH levels (6, 7, 8, and 9) on *E. acoroides* seedling growth over 8 weeks in controlled aquariums. All treatments showed 100% seed germination during the first week. By week 2, no significant differences in biomass were observed, but by week 8, seedlings at pH 6 had the highest dry weight (0.21 ± 0.01 g), as well as the greatest leaf number (5.64 ± 0.15 leaves), leaf length (23.39 ± 2.06 mm), and leaf width (4.74 ± 0.14 mm). One-way ANOVA revealed significant differences in growth by week 8. Lower pH levels enhanced shoot and root growth, while higher pH increased root number but reduced root length. Chlorophyll content analysis showed no correlation with pH after 8 weeks. Anatomical examination revealed tannin cells, starch granules, and thick cell walls in the mesophyll, with an enlarged exodermis in lower pH treatments, suggesting an adaptation for stabilizing in muddy, acidic conditions. These findings indicate that pH influences the growth and adaptation of *E. acoroides* seedlings, highlighting the species resilience to acidification. Its adaptive capacity is crucial for management, as *E. acoroides* can survive acidification and continue providing habitat, preserving ecosystem balance.

KEYWORDS: *Enhalus acoroides*, seagrass anatomy, pH effect, tropical seagrass

INTRODUCTION

Seagrass meadows are one of the most important maritime shore ecosystems in the world. It offers food for humans, dugongs, sea turtles, fish, and other invertebrates. It also functions as a fish and shrimp nursery, a sediment trap, and a nutrient cycler. Furthermore, seagrass meadows relate to the coral reef ecosystem, which promotes coral reef productivity [1]. *Enhalus acoroides* (Linnaeus f.) Royle commonly known as tape seagrass, is the most extensive seagrass species found in Thailand [2]. It predominantly inhabits the lower intertidal and subtidal regions, with a maximum depth of approximately 7 meters [3]. Tape seagrass is used by coastal communities for various purposes. Its leaves, containing flavonoids, tannins, terpenoids, and saponins, are used as a natural mosquito repellent in Indonesia [4]. Indian communities incorporate seagrass into fertilizers, while in Australia and the Philippines, seagrass fibers are used in fishing equipment, and its seeds are eaten [5]. In Thailand, research is underway to create eco-friendly containers from seagrass fibers [6]. This species is resistant to fluctuations in temperature and nutrients. As a result, this species will react to changes in its environment through physiological, morphological, biochemical, and anatomical features, all of which can serve as indicators of environmental change [7].

Ocean acidification (OA) is recognized as a sig-

nificant global concern within the context of climate change [8]. The reduction in oceanic pH levels is attributed to the significant rise in atmospheric CO₂ levels, which subsequently dissolves into the ocean. The process of CO₂ dissolution leads to the formation of H₂CO₃, which subsequently dissociates into H⁺, thereby causing a reduction in the pH of the ocean [9]. OA significantly impacts the growth, development, survival, and calcification of marine organisms, with bivalves and gastropods being most affected, while cephalopods exhibit greater resilience [10]. These acidic conditions often fluctuate markedly in intertidal zones, where they directly influence seagrasses, an essential component of coastal ecosystems [11]. In the context of increasing atmospheric CO₂ levels, estuarine and coastal waters suffer more severely from acidification than the open ocean [12]. In response to acidification, primary producers such as seagrass often exhibit increased photosynthetic activity, which is linked to metabolic changes as well as morphoanatomical and ultrastructural alterations [13]. Additionally, elevated CO₂ concentrations have been shown to enhance photosynthesis, growth, survival and proliferation of eelgrass (*Zostera marina*) [14]. While pH increases, *Z. marina*, *Ruppia maritima* and one green macroalga are associated with a continuous decline in photosynthetic rates [15]. However, the specific anatomical alterations resulting from pH fluctuations remain poorly understood. Furthermore, the response

of seagrass to changing pH varies between species, with distinct species-specific differences in their adaptations to acidification.

CO₂ alteration has a direct impact on plant photosynthetic rates as well as biochemical activities in seagrasses. Such fluctuations can cause morphological and physiological changes in seagrasses, similar to the consequences of soil pH changes in terrestrial plants. Few studies have investigated the underlying anatomical structures of different seagrass species, including *Halodule wrightii* leaves, and how these structures may be altered in response to ocean acidification. A previous study discovered that *H. wrightii* could adapt to acidic environments in the ocean [13]. *Posidonia oceanica* (L.) Delile and *Cymodocea nodosa* (Ucria) Ascherson were investigated for the effect of pH on photosynthesis, which revealed a linear decline in photosynthetic rates at pH 8.8. However, *Zostera noltii* exhibited markedly reduced sensitivity to pH, demonstrating a significant drop only at pH 9 [16]. The impact of ocean acidification on *E. acoroides* leaves was also investigated, and it was found that *E. acoroides* increased its rate of carbon fixation in response to a decrease in pH. The findings indicate that tape seagrass demonstrates great potential for carbon sequestration, which is an important component in the restoration of seagrass meadows within the scope of environmental governance [17]. However, the previously mentioned studies assessed the impact of acidification on fully grown seagrasses. Currently, there is a lack of research examining the impact of reduced pH levels on the growth and anatomical characteristics of tape seagrass seedlings. Related studies have shown that HCl-induced seawater acidification produced clear metabolic effects in *Mytilus edulis*, which were comparable to those observed under HCO₃⁻-induced acidification [18]. Investigating the influence of HCl-induced pH changes on seagrass seedlings could provide valuable insights into the developmental responses of this species. It may also give the way for further studies, such as histochemical analyses, to better understand how low pH affects seagrass growth at the anatomical level. The aim of the present study is to investigate the effect of pH on the growth and anatomical characteristics of *E. acoroides* seedlings. This study is crucial for predicting the future of seagrass ecosystems, which are essential for maintaining biodiversity, supporting coastal protection, and mitigating climate change effects.

MATERIALS AND METHODS

Plant materials

E. acoroides fruits were collected at low tide from the intertidal area at Paklok Bay, Phuket, Thailand. Fruits were measured between 25–35 mm in diameter. For each fruit, seeds were collected and washed with seawater. The experiments were performed within

24 h after collection from the field.

Experimental design

A total of 360 seeds of *E. acoroides*, each with a diameter of 10 mm, were cleaned of epiphytes and kept in aerated 10-l glass tanks containing 6 l of air-bubbled natural seawater which was obtained from the Phuket Marine Biological Center (PMBC) and filtered through Whatman GF/C filters. This study used a completely randomized design (CRD) with a total of 120 seeds that were transferred to various seawater pH levels, including pH 6, 7, 8 and 9, under controlled temperature (25 ± 1 °C), saturating irradiance (light:dark; 12 h:12 h) and air-bubbled natural seawater ($n = 30$ per treatment). pH 6, 7, 8 (control), and 9 were measured by a pH meter (Thermo Fisher Scientific, Massachusetts, USA) adjusted by adding appropriate quantities of 1 N NaOH or 1 N HCl every day at the same time to obtain incubation media with pH values at intervals of approximately ±0.2 units. Temperature, pH and irradiance were monitored in all treatments during the experiment. Seedlings were cultured for 2 months. After a 2-month of acclimation period under control conditions, *E. acoroides* seedlings were transferred to the treatments. For growth and development analysis, five seedlings were sampled from each treatment every two weeks over an 8-week period. Chlorophyll content was assessed in week 4 using three seedlings per treatment. Anatomical observations were conducted at week 8, with three seedlings per treatment used for freehand sectioning and an additional three for paraffin sectioning.

Growth and development of *E. acoroides* seedling under different pH conditions

Leaf number, leaf length, leaf width (5 mm from the leaf tip), root number (5 mm from the root tip), root length, fresh weight, and dry weight were collected every 2 weeks for 8 weeks.

Anatomical study of *E. acoroides* seedling under different pH conditions

For the anatomical investigation by freehand section, leaves and roots from each treatment ($n = 3$ per treatment) after being cultured for 2 months were collected and cut into smaller pieces (2 cm from the apex) with a razor blade to evaluate epidermal cells. This procedure involves investigating leaf surface cell characteristics. First, a 0.5 cm section of the third leaf was cut at 2 cm from the apex and placed on a glass slide to create a wet mount with distilled water. Photographs were acquired with an Olympus BX-51 microscope and DP28 digital camera (Olympus Corporation, Tokyo, Japan) to determine epidermal cell anatomy. Cross-sections of leaf tips were obtained (2 cm from the apex) to indicate structural elements such as the upper and lower epidermis, vascular tissues, and air lacunae (aerenchyma).

For the anatomical investigation by paraffin section, the 5-mm-thick mature leaves (2 cm from the apex) and roots (1 cm from the root tip) ($n = 3$ per treatment) were fixed in FAAI (formaldehyde, glacial acetic acid, 70% ethyl alcohol, 5:5:90 (v/v/v)) for 48 h and washed with 70% ethanol three times for 3 h each. The samples were dehydrated using a graded ethanol-tertiary-butyl alcohol series (70%, 85%, 95%, and 100%), then infiltrated with liquid paraffin and embedded in paraffin wax (paraplast) (Leica Biosystems, Nussloch, Germany). Samples in paraffin blocks were sliced into 6- μ m-thick slices with a rotary microtome. The sections were mounted on microscope slides and stained with safranin and fast green [19]. Photographs were acquired with an Olympus BX-51 microscope and DP28 digital camera (Olympus Corporation, Tokyo, Japan). Anatomical parameters were measured using ImageJ software in 3 replications per plant. For each anatomical parameter, measurements were taken from 3 plants per treatment. From each plant, 6 microscopic fields were randomly selected per slide, with 1 representative cell measured per field, resulting in a total of 18 measurements per treatment.

Chlorophyll content of *E. acoroides* seedling under different pH conditions

Mature leaves (0.1 g fresh weight) of *E. acoroides* from different pH conditions were crushed in 5 ml of 80% cooled acetone at room temperature and under dark conditions ($n = 3$ per treatment), then centrifuged at 3,000 rpm for 10 min. The supernatant was stored in a dark, cool place. UV-visible spectroscopy (HP 8453E, Santa Clara, CA, USA) was used to measure the supernatant at 480, 510, 645, and 663 nm respectively. The amounts of chlorophyll a, chlorophyll b, total chlorophyll, and carotenoid contents were computed using the formula below:

$$\begin{aligned}\text{Chlorophyll a} &= [12.7(A_{663}) - 2.63(A_{645})] \times V / (1000 \times \text{FW}); \\ \text{Chlorophyll b} &= [22.9(A_{645}) - 4.68(A_{663})] \times V / (1000 \times \text{FW}); \\ \text{Total chlorophyll} &= [20.2(A_{645}) + 8.02(A_{663})] \times V / (1000 \times \text{FW}),\end{aligned}$$

where A480, A645, A510, and A663 are the absorbance at 480, 645, 510, and 663 nm (V: extraction volume (ml), FW: fresh weight (g) [20].

Statistical analysis

The results were expressed as mean \pm S.E. Data were statistically analyzed by one-way analysis of variance (ANOVA) using SPSS® Statistics software version 20. The level of significance for all statistical tests was $p < 0.05$.

RESULTS AND DISCUSSION

Growth and development of *E. acoroides* seedling

Although genetic variation might affect seed germination and early plant growth, this study minimized such effects by using seeds from the same fruit and selecting uniform fruit and seed sizes to ensure genetic consistency. In natural conditions, *E. acoroides* seeds typically

germinate rapidly under favorable environments [17] and our findings confirm that they germinate well under controlled conditions, thereby allowing for a clear interpretation of treatment effects. In this study, HCl was used to lower the pH, introducing Cl^- ions that are largely inert in seawater and unlikely to affect photosynthetic rates. Nevertheless, this method effectively increased H^+ concentration, allowing us to examine its direct effects on *E. acoroides*. To our knowledge, this is the first report describing the anatomical responses of this species to pH variation.

Under various pH levels, seagrass seeds germinated 100% in all treatments within the first week, with the radicle and first leaf in all seeds (Fig. 1). The result indicated that changes in pH do not affect the germination of seagrass seeds when compared to the control (pH 8). This could be because the mechanism of seed germination depends on a wide range of parameters, including both internal factors like the nutrients stored in the endosperm [21] and external variables like temperature and dissolved oxygen [7]. Germination may be triggered by the starch that is deposited at the seed base [22]. The findings also demonstrated that *E. acoroides* seedlings rely on their own internal energy stores. Additionally, the change in pH in seawater, which has a buffering capacity, can fluctuate and reduce acidity levels throughout the day [23]. This buffering capacity can mitigate the toxic effects of pH changes on the germination of seagrass seeds in this experiment.

All germinated seedlings survived throughout the 8-week experimental period under all pH treatments, resulting in a 100% survival rate. After two months of growth with measurements taken every two weeks, distinct growth patterns were observed. Radicles, root hairs, and leaves were presented in all treatments; however, no root hairs at the seed base were found at pH 6. By week six, all treatments had produced a second root (embryogenic root) (Fig. 1). Root hairs were visible throughout, from pH 7 to pH 9. From the observation of the growth pattern, seedling development of *E. acoroides* is dependent on the pH of the water [23]. The root hairs observed at the seed base of *E. acoroides* may function similarly to the root hair-like filamentous cells found on the basal surface of *Thalassia hemprichii* seeds, which serve as anchoring structures prior to primary root development. These structures facilitate initial attachment to sediments, providing stability and leverage necessary for the primary roots to penetrate the substrate [22]. Therefore, acidic conditions may disrupt this anchoring system, potentially preventing successful attachment to the substrate and increasing the risk of seagrass loss.

Growth of seagrass was assessed every 2 weeks, separating measurements into fresh weight (FW) and dry weight (DW) of the aboveground parts, including leaves and seeds, and the belowground parts, including roots. The goal was to determine the influence of

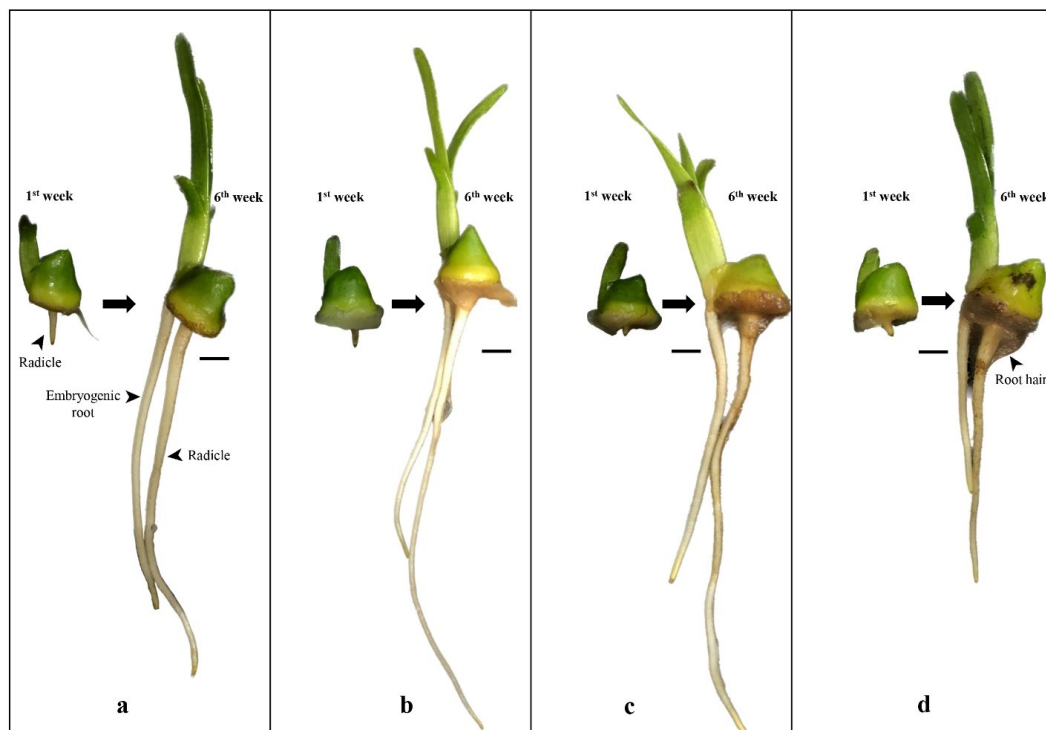


Fig. 1 The development of seagrass seeds grown under different pH conditions (a = pH 6, b = pH 7, c = pH 8 control, d = pH 9) bar = 0.5 cm.

pH changes on different parts of the *E. acoroides*. There were no significant differences between pH treatments in aboveground FW (Fig. 2a) at weeks 2, 4, and 6. However, at week 8, seagrass growing at pH 6 had significantly higher FW than the control at pH 8 (Fig. 2b). This result was similar to belowground FW, which was not significantly different in weeks 2 and 4, although pH 6 and pH 7 were significantly higher than the control in weeks 6 and 8, respectively. However, pH 9 resulted in significantly lower FW than the control. This pattern was consistent with the increase in aboveground DW (Fig. 2c,d), indicating that the reduction in pH led to increased leaf growth, possibly due to an increase in the rate of photosynthesis [17]. The results are related to those obtained in *E. acoroides* leaves, which indicated that it has a great survival ability and a high capability to resist OA [17]. Furthermore, the statistically significant difference observed in week 8 suggests that in the early stages of growth, differences in pH may not have a significant impact, possibly due to the use of energy from the endosperm and the seagrass ability to adapt well to its environment.

From the growth results, it can be observed that lowering the pH improves the aboveground growth of *E. acoroides*. Therefore, the leaf growth of the seagrass was evaluated by counting the number of leaves and measuring the size of the leaves, including their length and width, at a position 0.5 cm from the tip of the

longest leaf, specifically focusing on the 4th leaves. In terms of the number of leaves, there was no significant difference in the number of leaves between weeks 2–4. However, a significant difference was observed in week 8, with pH 6 showing the significantly higher in number of leaves and leaf length than the control (Fig. 3a,b). However, there was no significant difference in leaf width among treatments in 2, 4, 6 and 8 weeks observed (Fig. 3c). Over an 8-week period, root number and length were evaluated at different pH levels. The root number showed a progressive rise but no significant differences between treatments in weeks 2, 4, and 6 (Fig. 3d). In week 8, pH 6 produced a much higher root number than the control (pH 8). Surprisingly, by week eight, pH 9 yielded the highest root number (Fig. 3d). The longest roots, measuring around 75 mm by week 8 (Fig. 3e), were produced at pH values 6 and 7, and the length of the roots increased dramatically over time. Compared to other treatments, roots grown at pH 9 at week 8 have shorter lengths. In the process of root elongation, the auxin hormone plays a crucial role by stimulating the activity of H^+ -ATPase proteins on the plasma membrane. These proton pumps extrude H^+ ions into the apoplast, thereby lowering the local pH and creating an acidic environment that activates expansion proteins, a mechanism known as the acid growth theory, which facilitates cell wall loosening and promotes root elonga-

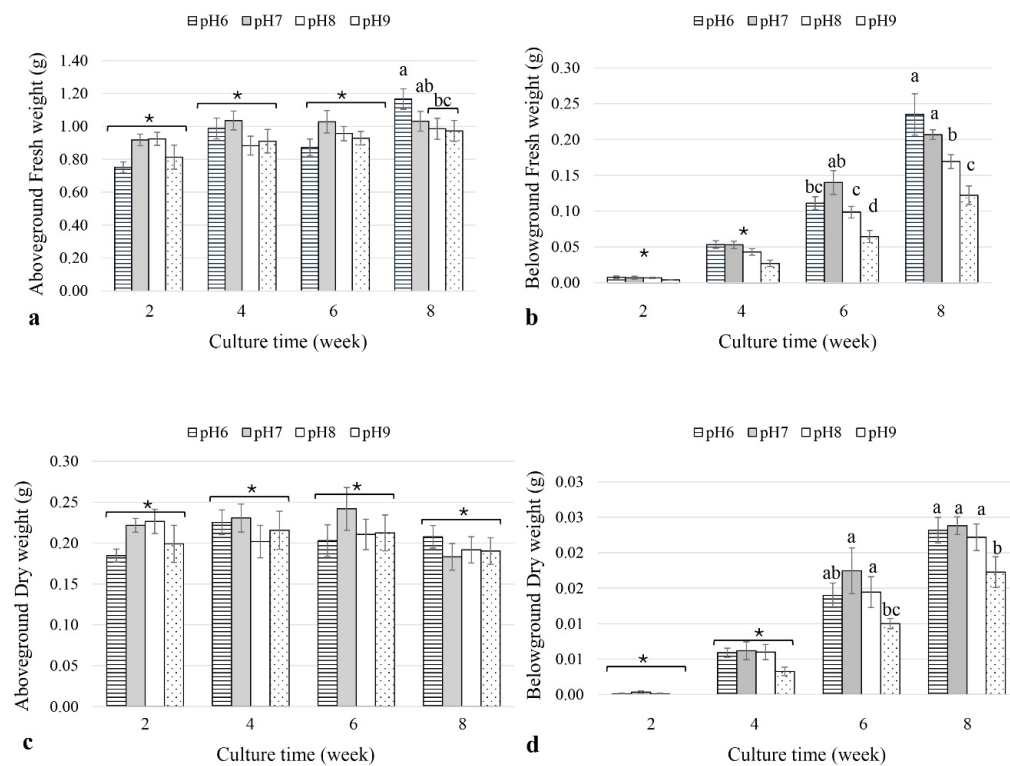


Fig. 2 Aboveground (a) and belowground (b) fresh weight, and aboveground (c) and belowground (d) dry weight of *E. acoroides* grown under different pH conditions. Error bars represent the standard error. Different letters indicate significant differences between treatments.

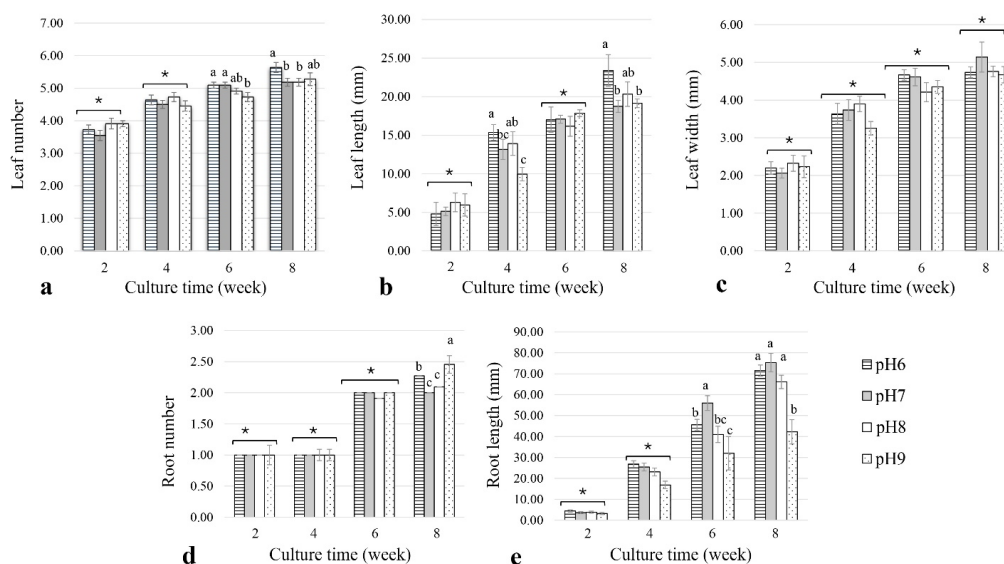


Fig. 3 The impact of water pH on leaf number (a), leaf length (b), leaf width (c), root number (d) and root length (e) of *E. acoroides* seedling. Error bars represent the standard error. Different letters indicate significant differences between treatments.

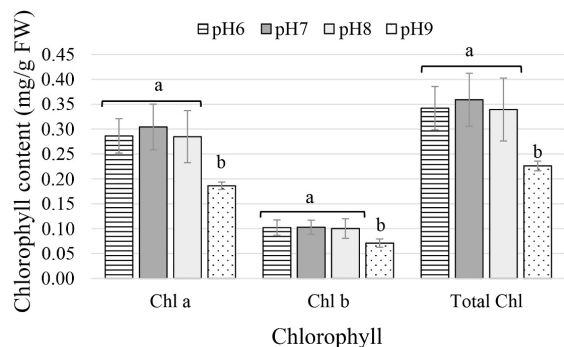


Fig. 4 The impact of water pH on photosynthetic contents of *E. acoroides* seedling at week 4. Error bars represent the standard error. Different letters indicate significantly different.

tion [24]. In addition, increased external acidity may also contribute to enhanced apoplastic acidification, thereby reinforcing cell wall loosening and supporting root growth. This may explain why roots were longer at lower pH levels (6–8) compared to pH 9.

Although this experiment showed successful growth of *E. acoroides* with well-developed roots and rhizomes, several environmental factors in natural conditions can affect seedling establishment, such as sedimentation. Seedlings are strongly influenced by the substrate type, preferring fine sand and low wave exposure [17]. Since natural seeds germinate immediately after fruit release, this stage is critical for anchoring and growth. Other factors include sediment disturbance. It has been reported that seeds of *Zostera nigricaulis* survive better when placed on the sediment surface compared to being buried 1–2 cm deep in the sediment. This may be due to limited access to key germination factors such as oxygen and light when seeds are buried [25].

Chlorophyll contents responses to various pH

Significant differences in chlorophyll a, b and total chlorophyll were found only week 4. *E. acoroides* growing in seawater pH 9 showed the significantly lowest of chlorophyll content (Fig. 4), however, no differences were observed at week 8. Seagrasses differ from terrestrial plants in that high pH levels are likely to restrict photosynthesis, which leads to a decrease in the pigments required by the process. For terrestrial plants, a drop in soil pH usually results in reduced nutrients uptake [26]. A lower pH, however, enhances the availability of CO_2 in seagrasses, both in free form and as HCO_3^- . Both forms can be used as photosynthetic substrates by *E. acoroides*. This is especially helpful because the ocean naturally contains less CO_2 than acidic soils, where terrestrial plants are negatively impacted by decreased pH. As a result, there was no apparent distinction between the pH 6, pH 7 and pH 8

on *E. acoroides*. However, this study is different from others that have shown higher rates of photosynthesis in seawater with higher acidity. The difference can result from variations in the developmental phases that were examined on. In this case, the study concentrated on seedlings, whose growth is partially dependent on stored energy. This study also discovered that the amount of chlorophyll was considerably decreased by increased pH values. This might be the consequence of changes in nutrient intake brought on by pH, which would eventually inhibit growth.

In seagrasses, lower pH enhances the availability of inorganic carbon, both as free CO_2 and bicarbonate (HCO_3^-), which facilitates photosynthesis [16]. *E. acoroides* can utilize both forms as photosynthetic substrates. This is particularly important because seawater naturally contains less free CO_2 than acidic soils, where terrestrial plants tend to be negatively affected by low pH. Our findings are also consistent with previous research on *C. nodosa*, which showed a slight decrease in chlorophyll at pH 7.5 [27]. Fluctuations from neutral pH, whether acidic or alkaline, may affect chlorophyll synthesis depending on the species and its developmental stage. In this study, chlorophyll reduction under high pH may reflect impaired nutrient uptake, limiting seedling growth and photosynthetic efficiency.

Anatomical study of *E. acoroides* seedling affected by various pH conditions

The study found that the epidermal cells of *E. acoroides* leaves were rectangular, approximately 30 μm in width and 60 μm in length, with clearly defined cell wall boundaries (Fig. 5). Based on visual estimation, chloroplast abundance in *E. acoroides* appeared highest at pH 6 (Fig. 5a), followed by pH 7 (Fig. 5b) and the control at pH 8 (Fig. 5c). At pH 9, the leaves started turning brown, and the cell membranes shrank (Fig. 5d, arrow), making the boundaries between the cell membrane and the cell wall more distinct compared to seagrasses maintained at other pH levels. This could be due to the lower pH levels providing more available CO_2 for photosynthesis, allowing for higher starch production. In contrast, increasing the pH reduces the dissolved CO_2 levels, leading to a decrease in observable chloroplasts. Furthermore, the increase in pH could make seagrass more susceptible to mortality. This finding agrees with the measured chlorophyll content (Fig. 4), which could be due to the high adaptability of seagrass, allowing it to regulate and maintain a stable photosynthesis rate. Moreover, the increased chloroplast density in epidermal cells, allowed them to capture light that is partially obscured by water.

Cross sections of *E. acoroides* leaves revealed that chloroplasts were densely packed within both the upper and lower epidermal cells (Fig. 6a,d,g,j). The mesophyll did not show clear differentiation into pal-

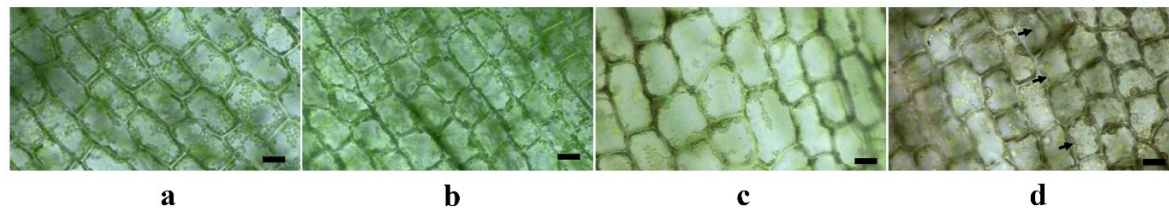


Fig. 5 Epidermal cells of *E. acoroides* leaves grow in different pH conditions including pH 6 (a) pH 7 (b), control pH 8 (c) and pH 9 (d) (bar = 20 µm). Arrows indicated shrunken cell membranes.

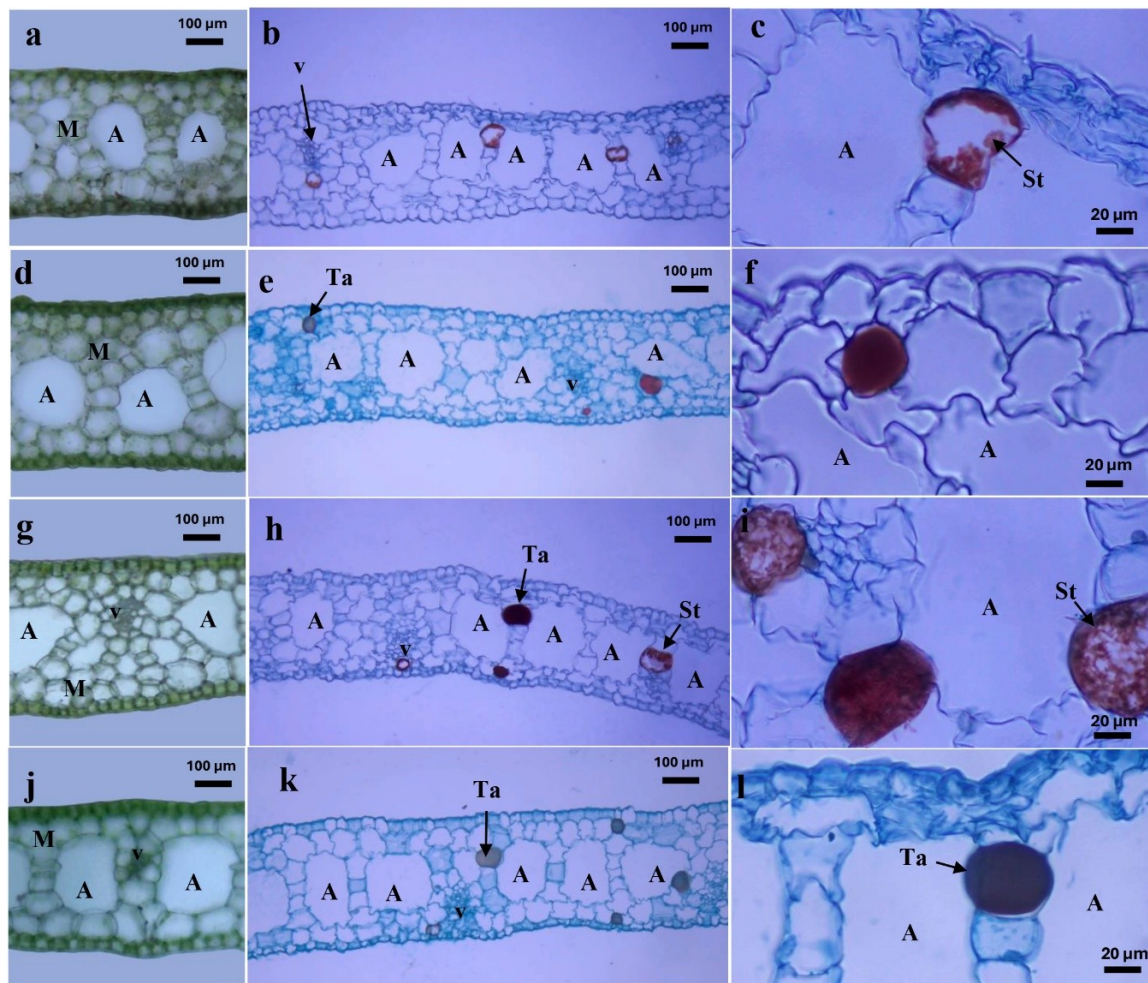


Fig. 6 Cross section of leaf of *E. acoroides* grow in different pH conditions. a–c = pH 6, d–f = pH 7, g–i = pH 8, j–l = pH 9, v = vascular bundle, Ta = tannin cell, St = starch granules, A = air lacunae.

isade and spongy layers and was therefore identified simply as mesophyll tissue (M). However, air lacunae (A) were present (Fig. 6a,d,g,j). Similar structures were observed in samples prepared using the paraffin method, which provided clearer visualization of the leaf anatomy (Fig. 6b,e,h,k). Tannin cells (Ta) and starch granule-containing cells (St) were also distinctly distributed throughout the mesophyll, especially near

the air lacunae, and were stained dark or light brown with safranin (Fig. 6c,f,i,l).

Samples exhibited similar tissue arrangements on both abaxial and adaxial surfaces and lacked stomata, a trait also observed in other seagrass species [28] such as *Phyllospadix iwatensis* [29]. *E. acoroides* lacked stomata and cuticles at all pH levels, consistent with previous studies that reported the absence of stomata

in seagrasses [30]. In contrast to terrestrial plants, where chloroplasts are mainly located in mesophyll cells, chloroplasts in *E. acoroides* were found in epidermal cells. Chloroplasts were discovered to be dispersed throughout the epidermal cells of *E. acoroides* leaves, which had a basic structure without stomata or hairs. Similar morphological adaptations were observed in seagrasses from Trang Province, supporting the idea that the epidermis serves as the primary site of photosynthesis in this species [31]. This could be attributed to the formation of air lacunae in the mesophyll cells. Seagrasses may adapt by floating closer to the ocean surface to increase light and CO₂ availability, which could result in larger and more numerous air lacunae. Our study indicated that seagrass leaves produce tannin, accumulating in the mesophyll cells of leaves at every pH condition tested. Tannin is typically produced in vacuoles and is classified as a secondary metabolite. Generally, these substances do not have a specific function or play a critical role in plant growth. They mostly function as a defense mechanism against pathogens and herbivores [31]. The presence of tannins may still play a role in their remaining self-defense strategies that have evolved over time. In *Thalassia testudinum*, the production of tannin could serve to protect against excessive grazing by various marine animals [32]. Thus, tannin accumulation might contribute to the self-defense mechanism of *E. acoroides* and may also contribute to structural resilience for survival in seawater environments. The accumulation of tannins in the leaves of *E. acoroides*, as observed in this study, is consistent with the findings of Shaffai et al [33], who reported that phenolic compounds, including tannins, are most abundant in the leaves compared to other plant organs. This suggests a potential defensive role of tannins against herbivory, particularly in young leaves, and reflects an evolutionary adaptation of seagrasses in response to herbivores under marine environmental pressures.

Measurements of parameters associated with the anatomical features of leaves of *E. acoroides* growing in various pH conditions are shown in Table 1. Adaxial epidermal cell thickness was highest at pH 7, followed by the control, while values at pH 6 and 9 were lower. This suggests that neutral to slightly alkaline (pH 7 and 8) may support epidermal cell development more effectively than more extreme pH levels. The abaxial epidermis thickness was greatest at pH 6, and gradually declined with increasing pH, reaching its lowest at pH 9. Compared to the control, pH 6 enhanced the development of the lower epidermis, while pH 9 appeared to suppress it, suggesting that both highly acidic and alkaline environments may inhibit epidermal cell development.

For mesophyll thickness, pH 7 promoted the greatest development, exceeding the control, while values declined at both pH 6 and 9. This pattern indicates that mesophyll tissue may be particularly sensitive to

deviations from neutral pH, with pH 9 having the most inhibitory effect. These results align with Schneider et al [13], who found that elevated acidity induces leaf thickening, potentially as an adaptive response. Overall, pH 7 resulted in enhanced anatomical development across most parameters compared to the control, while pH 9 consistently showed reductions, highlighting its potential negative impact on leaf structure. The number of air lacunae decreased under all pH treatments compared to the control, with the sharpest decline at pH 6. Additionally, lacunae size became smaller as pH increased. These findings suggest that larger air spaces at neutral pH may facilitate better gas and nutrient exchange [34]. This aligns with Mee-sawat et al [30], who reported that *E. acoroides* adjusts its internal anatomy, including air lacunae formation, in response to environmental changes.

Root anatomy

The root structure of *E. acoroides* consists of an epidermis with root hairs (Rt) emerging from epidermal cells, followed by an exodermis (Ex) (Fig. 7a,c,e,g). The exodermis, often containing lignin or suberin, helps regulate water and mineral uptake [29]. The cortex comprises parenchyma cells, though no air lacunae were found at this stage. The stele includes the xylem and phloem, but lignin was not detected in the xylem region. Starch granules (St) and tannin cells (Ta) were present across all treatments (Fig. 7b,d,f,h).

Anatomical structures at pH 6, pH 7, and the control (pH 8) showed similar characteristics. In contrast, pH 9 induced distinct changes, including reduced root hair density, increased tannin cell abundance, and a less distinct exodermal boundary. These changes suggest that highly alkaline conditions may disrupt proton dynamics, consistent with the role of pH gradients and H⁺-ATPase activity in root hair development [35]. Moreover, plant nutrient uptake has been reported to decrease markedly with increasing soil pH [36], possibly due to a reduction in root hair formation.

CONCLUSION

This study highlights the remarkable adaptability of *E. acoroides* seedlings to varying pH levels, with germination unaffected by acidity and achieving a 100% success rate across all treatments. Optimal growth was observed at pH 6 and 7, where enhanced CO₂ availability likely drove significant increases in root and leaf development. Anatomical studies revealed that leaves across treatments lacked stomata, had a thin cuticle, and utilized their epidermis as a major photosynthetic site, with reduced water-conducting elements. Additionally, acidic conditions promoted resilience through increased chloroplast density and air lacunae adaptations. While the root structure remained consistent at pH 6, 7, and the control (pH 8), pH 9 roots exhibited smaller root hairs, abundant tannin cells, and an undefined exodermis, indicating developmental impair-

Table 1 Parameters associated with the anatomical features of leaves of *E. acoroides* growing in various pH conditions.

Parameter (Average ± S.E.)	pH 6	pH 7	Control (pH 8)	pH 9	F	p-value
Leaf x.s.						
Adaxial epidermis thickness (µm)	16.62 ± 0.74 ^b	23.68 ± 1.3 ^a	21.65 ± 1.01 ^a	17.15 ± 1.31 ^b	9.549	0.000
Mesophyll thickness (µm)	180.41 ± 4.68 ^c	222.39 ± 6.37 ^a	200.40 ± 3.51 ^b	192.49 ± 7.21 ^{bc}	9.851	0.000
Abaxial epidermis thickness (µm)	19.90 ± 1.84 ^a	17.33 ± 0.73 ^{ab}	15.35 ± 0.61 ^b	16.03 ± 0.64 ^b	3.433	0.022
Air lacunar area (µm ²)	13,232.0 ± 544.6 ^a	13,848.8 ± 1,456.9 ^a	14,478.8 ± 702.0 ^a	10,639.8 ± 766.6 ^b	3.657	0.017
Air lacunar number (no/mm ²)	29.28 ± 0.87 ^a	20.37 ± 0.54 ^b	19.31 ± 0.74 ^b	28.06 ± 2.40 ^a	14.363	0.001
Tanin	+	+	+	+		

Values with different letters within each row are significantly different (Duncan multiple range test by SPSS program version 20), S.E. = standard error.

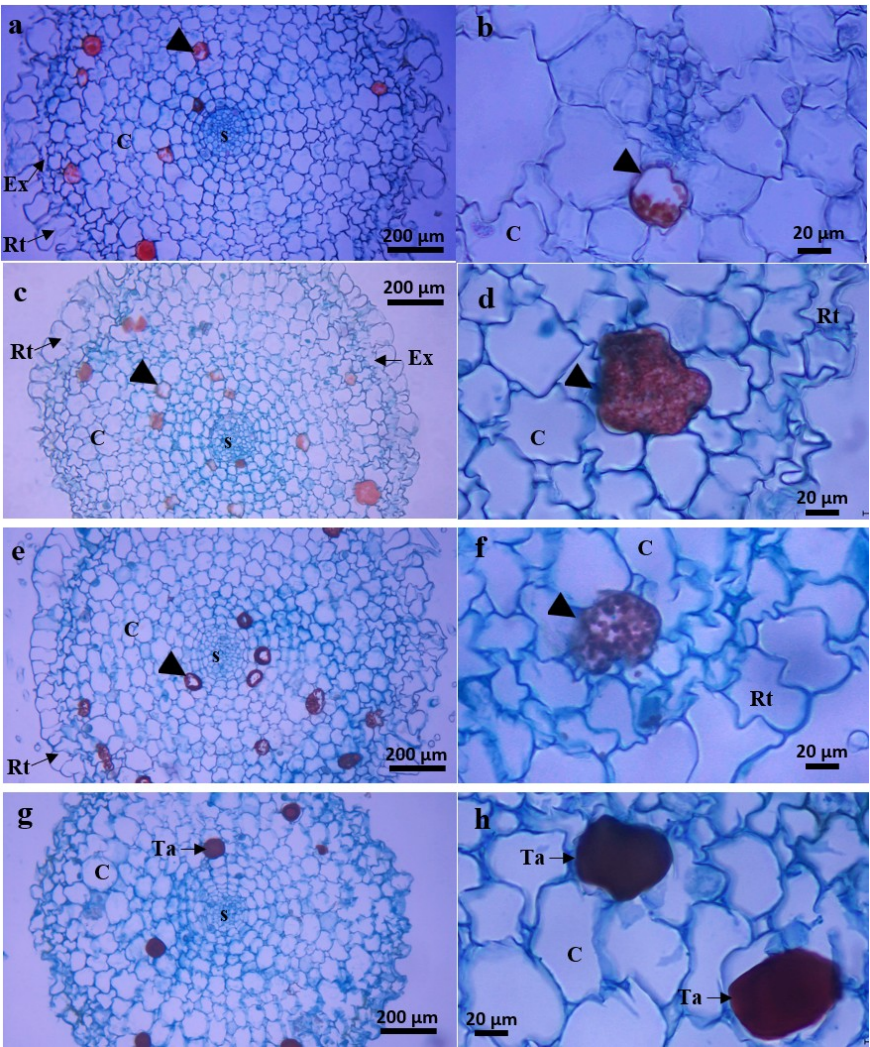


Fig. 7 Cross section of root of *E. acoroides* grow in different pH conditions pH 6 (a,b), pH 7, (c,d), control pH 8 (e,f) and pH 9 (g,h), st = starch granule, s = stele, ta = tannin cell, c = cortical cells, ex = exodermis, arrowhead = starch granule.

ment. Despite reduced biomass and chlorophyll content at pH 9, the seedlings demonstrated structural and metabolic flexibility by relying on internal energy reserves to maintain growth. These findings underscore the acid tolerance of *E. acoroides* and its potential role in supporting ecosystem resilience under future ocean acidification scenarios. Although *E. acoroides* is not a primary food source for dugongs to the same extent as *Halophila ovalis*, preserving biodiversity, including structurally and functionally diverse seagrass species remains critical to maintaining ecosystem stability and achieving sustainable marine conservation.

Acknowledgements: This work was supported by the Faculty of Technology and Environment, Prince of Songkla University, Phuket Campus and grants provided by the Electricity Generating Authority of Thailand (EGAT) under Grant number TAE6405050M.

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