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Seed Germination and Seedling Development of the Seagrass *Enhalus acoroides* (L.f.) Royle *in vitro*: Effects of Burial Depths and Desiccation Periods

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ABSTRACT

This study provides information on the feasibility of using *Enhalus acoroides* seeds as source of seedling materials for seagrass restoration effort and to support wider conservation initiatives. Mature seeds of *E. acoroides* were collected from Tubajon, Laguindingan, Misamis Oriental and were subjected to different laboratory conditions, namely burial depths and desiccation periods, to determine germination rate, seedling growth, and development. Seeds placed just above (control) and half-buried in the sediment fully germinated on the first day, while germination of fully buried seeds was delayed, attaining 93.3% germination on the fourth day. Seeds planted at 5 mm burial depth started germinating on the fifth day with only 56.7% germination success, while seeds buried deeply (15mm) did not germinate. All seeds subjected to desiccation germinated, but higher seedling survival (93.3 - 100%) was observed when seeds were desiccated at shorter periods of up to two hours while the lowest seedling survival (64.8%) was observed after a 12h desiccation period. One-way ANOVA showed significant differences in seedling survival and shoot length ($p<0.05$) but differences in moisture loss and germination rates were not significant ($p>0.05$). Results have demonstrated that seed germination and the subsequent seedling development were adversely affected by burial depths and desiccation periods. Seedlings at 5mm burial depth and 12h desiccation period exhibited black and white spots, curled and yellowish leaves, stunted growth, and high mortality rates.

Keywords: Tropical eelgrass, leaf morphology, culture, propagules

INTRODUCTION

Seagrasses have been receiving worldwide attention because of their multiple ecological functions in estuarine and coastal ecosystems, serving as habitat, nursery, and breeding grounds, and food sources for various marine organisms (Fortes, 1990). Seagrass beds support diverse faunal assemblages (Balestri *et al*., 1998) and inshore invertebrate and finfish fisheries. Unfortunately, many seagrass areas have been permanently lost due to coastal, commercial, and recreational developments. These issues have led to recent efforts to restore and conserve these ecosystems (Fortes, 1990; Stafel *et al*., 1997).

Enhalus acoroides is the tallest among seagrasses with large and thick rhizomes bearing long and wide leaves and produce flowers several times a year. Fruits of this seagrass range from 5-7cm long and seeds are 11.5cm long. The large sexual propagule allows this species greater capacity to recover after a disturbance (Phillips and Meñez, 1988; Rollon, 1998).

The maintenance and recovery of existing seagrass beds, as well as the establishment of new ones, depend on successful sexual reproduction and vegetative rhizome growth and branching. Seed production, and those events related to this process such as flowering, seed release, seed dispersal, seed germination and subsequent growth of seedlings, serve not only as a means of maintaining genetic diversity but also in the recruitment and re-establishment of new seagrass beds (Duarte *et al.,* 1997).

Enhalus belongs to a group of seagrass with seeds characterized by a membranous coat without a distinct dormancy period (Kuo and Kirkman, 1996). Not much information is available on seed germination of *E. acoroides* but some lessons can be learned from similar work on other seagrass species. The seeds of *Halophila ovalis* started germinating 3-4 weeks after collection, and after two months about 12% of the 1250 seeds germinated at various stages of seedling development (Kuo and Kirkman, 1992). Seeds of the eelgrass *Zostera marina* planted in 5mm of sediment and supplied with continuously running seawater showed lower germination success than seeds buried at 15 mm and 25 mm (Moore *et al*., 1992).

The experimental evaluation of the vertical growth response of seagrases to sand burial was studied by Terrados (1997) both in the laboratory for *Cymodocea nodosa* and in field experiments for *Thalassia hemprichii, Cymodocea serrulata, Cymodocea rotundata, Halodule uninervis* and *Syringodium isoetifolium*. Burial of seagrasses causes mortality of shoots, but those which survived grow faster by an increase in the number of leaves produced, the length of the vertical rhizome internodes, the length of the leaf sheaths, and faster growth rates of the vertical rhizomes. *T. hemprichii* and *H. uninervis* also showed an increase in vertical rhizome growth when buried in sand. Burial depth below 4 cm of sand has been shown to promote the greatest vertical growth response in *C. nodosa* seedlings (Terrados, 1997). The rate of appearance of new leaves was slower in the buried shoots than in the meristem and the length of the leaf sheath was smaller in the control treatment than in buried seedlings.

This study was implemented to evaluate seed germination performance of *E. acoroides* as affected by burial depths and desiccation periods, and to compare the growth and development of the germinated seedlings in terms of survival rate, shoot morphology, and biomass. Information on the feasibility of using *E. acoroides* seeds as source of seedling materials will be vital for seagrass restoration efforts.

METHODS

Collection of Plant Materials

Mature fruits of *Enhalus acoroides* are dark green in color. A total of 30 mature fruits of *E. acoroides* were collected through handpicking during the lowest low tide from the intertidal area of Tubajon, Laguindingan, Misamis Oriental (127°27.5'N, 8°38.25'E). The collection site has extensive seagrass beds with an approximate area of 1.36 km^2 (Toring, 1999). Collected fruits were placed in a styrofoam box with minimal amount of seawater and transported from the field to the laboratory. In preparation for the burial depth and desiccation period experiments, mature fruits were transferred into a basin and washed several times with seawater to remove sand, rock particles, and other contaminants. Seeds were separated from the fruit capsule by pressing the capsules between the fingers. All the seeds were then thoroughly mixed in the basin before placing in the experimental containers.

Figure 1. Culture containers showing germinating seeds of *Enhalus acoroides*

Preparation of Experimental Set-up

The sandy substrata used in the culture experiments were collected in front of the MSU-IFRD laboratory at Naawan, Misamis Oriental. The sediments were vigorously washed with seawater to remove silt and other contaminants and afterwards placed in culture containers (4L plastic jars, 6cm tall) a week before planting the seeds to stabilize the sediments (Fig. 1). Seawater was added slowly in order not to disturb the sediments. Each container was labeled according to treatment and replicate of the various experiments. Ten seeds were randomly picked for each of three containers representing three replicates in each treatment for each experiment. The containers were then placed outdoors arranged in a Completely Randomized Design.

Effect of Burial Depth

The experiment on seed germination at different burial depths was conducted for a period of 40 days from October 22, 2002 until December 1, 2002. A total of 150 seeds were used in five treatments with three replicates laid out in a Completely Randomized Design (CRD) experiment. For the control, seeds were placed just above the surface of the sediments. The treatment with half -buried seeds was prepared by burying one-half part of the seeds into the sediment. In the zero mm treatment, the seeds were completely buried but just below the surface, while in the last two treatments, seeds were buried 5mm and 15mm below the sediment surface. After planting the seeds, seawater was slowly added up to the brim of the culture container taking care not to disturb the newly planted seeds and the sediments. Water management was done by replacing the water in the culture container with filtered seawater three times a week.

A seed was considered germinating when the first foliage leaf or cotyledon had emerged and from then on was considered a seedling. The number of germinated seeds was counted for the first ten days, and seed germination rate was calculated. The shoots were cleaned regularly by moderately rubbing the leaves between the fingers to remove attached sediments and epiphytes. The top layer of the sediment and walls of the jars were also cleaned by scraping and siphoning off the brown film of filamentous algae.

The period of seedling establishment was 40 days and counting of the seedlings was done every two days. Survival rates were based on the number of germinated seedlings that survived in each treatment. Documentation of the growth and development of the seeds and seedlings was also conducted by taking pictures of the various stages of seedling growth.

After 40 days of culture, the seedlings were carefully harvested by hand with the roots and leaves intact. The seedlings were washed with seawater and carefully cleaned by hand to remove the sediment and epiphytes. The shoots were measured for the following morphometric attributes (Uy *et al.,* 2001):

a) **Leaves:** number of leaves per shoot, leaf width per shoot (mm), and total leaf length (mm), which is the total leaf length per shoot measured from the insertion point of the leaf into the leaf sheath up to the tip of each leaf

b) **Shoots:** shoot length (mm)

c) **Seeds:** seed width (mm); seed length (mm)

d) **Roots:** number of roots per shoot; root diameter (mm); root length (mm), measured from the junction of the shoot or base of the seed, up to the end point of each root.

After the morphometric measurements were taken, the samples were sorted out as leaves, shoots, seeds, and roots, for biomass determination. The sorted samples were oven-dried for 48 hours at 50- 60°C and weighed using an analytical balance until constant weighed was obtained.

Effect of Desiccation Periods

The experiment on the influence of desiccation period on seed germination and development was conducted outdoors from October 26, 2002 to December 5, 2002. A total of 150 seeds were used representing five treatments and three replicates using a Completely Randomized Design (CRD) experiment. Seeds were air-dried at room temperature for 0.5, 2, 5, and 12 hours. Seeds that were not desiccated served as the control. After desiccation, ten seeds were planted in previously prepared culture jars with cleaned sediments and seawater, each container representing one experimental unit. Monitoring of germinated seeds and seedling growth and development followed the same protocol as in the burial depth study.

Statistical Analysis

Data on seed germination, survival, morphometrics, and biomass of the seedlings in different treatments in the two experiments were subjected to an analysis of variance (ANOVA) to determine effects of various treatments on different parameters. Where significant differences were detected, the Tukey's HSD was conducted to determine the source of variation among treatments means at 5% level of significance (Gomez and Gomez, 1984).

RESULTS

Seeds placed on top of the sediments and those partially buried obtained a germination rate of 100% in day 1. Seeds buried just below the sediment surface and those buried 5mm from the surface also germinated but at a lower germination rate of 93.3% and 56.7%, respectively. In these two treatments the seeds started germinating on Day 1 and completed the germination on Day 9, but on Day 10 seed mortality had started (Table 1). Seeds planted 15mm below the sediment did not germinate and manifested black and white spots at the surface of the sediments indicating decay.

Among the four treatment levels, the seeds buried 5mm below the sediment surface yielded the lowest survival rate (61.1%) of the germinated seedlings (Table 1). Burial depth greater than 5mm resulted in reduced seedling emergence, slower growth rate and development, and abnormal seedlings. Certain morphological changes were observed on seeds and seedlings after several days of germination. Black and white spots or molds began to appear on the seeds, seedlings, and surface of the sediment on the sixth day of culture in zero and 5mm treatment levels, accompanied by a foul odor. At the end of the 40-day period the seedlings at the 5mm depth were stunted, producing the shortest shoot length (65.1mm) and fewer, smaller, and shorter leaves and roots compared to seedlings growing in the other treatments (control, half-buried, and zero) (Table 1, Fig. 2). Similarly, aboveground (leaves, shoots) and belowground (seeds, roots) biomass of the plant parts were generally high in the control, half-buried and zero treatments, and significantly low in 5mm treatment (Fig. 3).

Table 1. Days of full germination, germination rates, survival, and shoot length of the seedlings from *E. acoroides* seeds subjected to different burial depths. Probability values of one-way ANOVA, written in boldface, indicate that treatment means are significantly different (p<0.05). Values with the same superscript letter indicate no significant difference (Tukey's HSD).

Treatment levels	**Days of full germination**	**Germination rates**	**% Seedling survival after 40 days**	**Shoot length (mm)**
Control	1st day	100.0^b	100.0^b	187.2^b
Half-buried	1st day	100.0^b	96.7^b	211.4^b
Zero	4th day	93.3^b	88.9^b	166.6^b
5 mm	9th day	56.7^a	61.1^a	65.1^a
15 mm	-	-	-	-
p values		**0.001**	**0.003**	**0.001**

All seeds subjected to different levels of desiccation in this study germinated (>96%) during the first day of experiment, together with the seeds that were not desiccated. No significant differences in germination rates were found among the two treatments (Table 2).

Desiccating the seeds up to 12h did not affect seed germination which may indicate that short term desiccation appeared not critical for seed germination. On the other hand, desiccation resulted in some physical alteration on seed quality. The seeds were shiny, dark green in color and hard before they were subjected to different desiccation periods. After desiccation, however, the seeds had shrunk, became a lusterless dark green and soft, and had brown spots at their bases. Seeds of *E. acoroides* desiccated for 12h obtained the highest moisture loss (56.7%) as shown in Table 2.

Significantly lower seedling survival rates were obtained from seeds desiccated at 5h (86.7%) and 12h (64.8%) compared to those from the other treatments (>93.3%, Table 2). These results indicate that seed desiccation can reduce the chance of successful seedling development in *E. acoroides.*

As observed in the burial depth experiment, black and white spots or molds appeared on the seeds, seedlings, and surface of the sediments in treatments subjected to 5h, and 12h seed drying on the fifth day of culture. At the end of the 40day period, seedlings from seeds subjected to 12h desiccation period appeared to be greatly affected by desiccation resulting in grossly inferior characters: short shoot length (75.8mm), fewer and smaller leaves and roots, shorter leaf length and root length, smaller leaf width, and root diameter. Some seedlings had no roots at all. Seedlings from 12h desiccation deteriorated and released a foul odor while leaves became curled and yellowish and had stunted growth (Table 2; Fig. 4). Biomass of above-ground and below-ground parts of the seedlings treated to 12h desiccation period was significantly low (Fig. 5) as compared to the other treatments (control, 0.5, 2, and 5h).

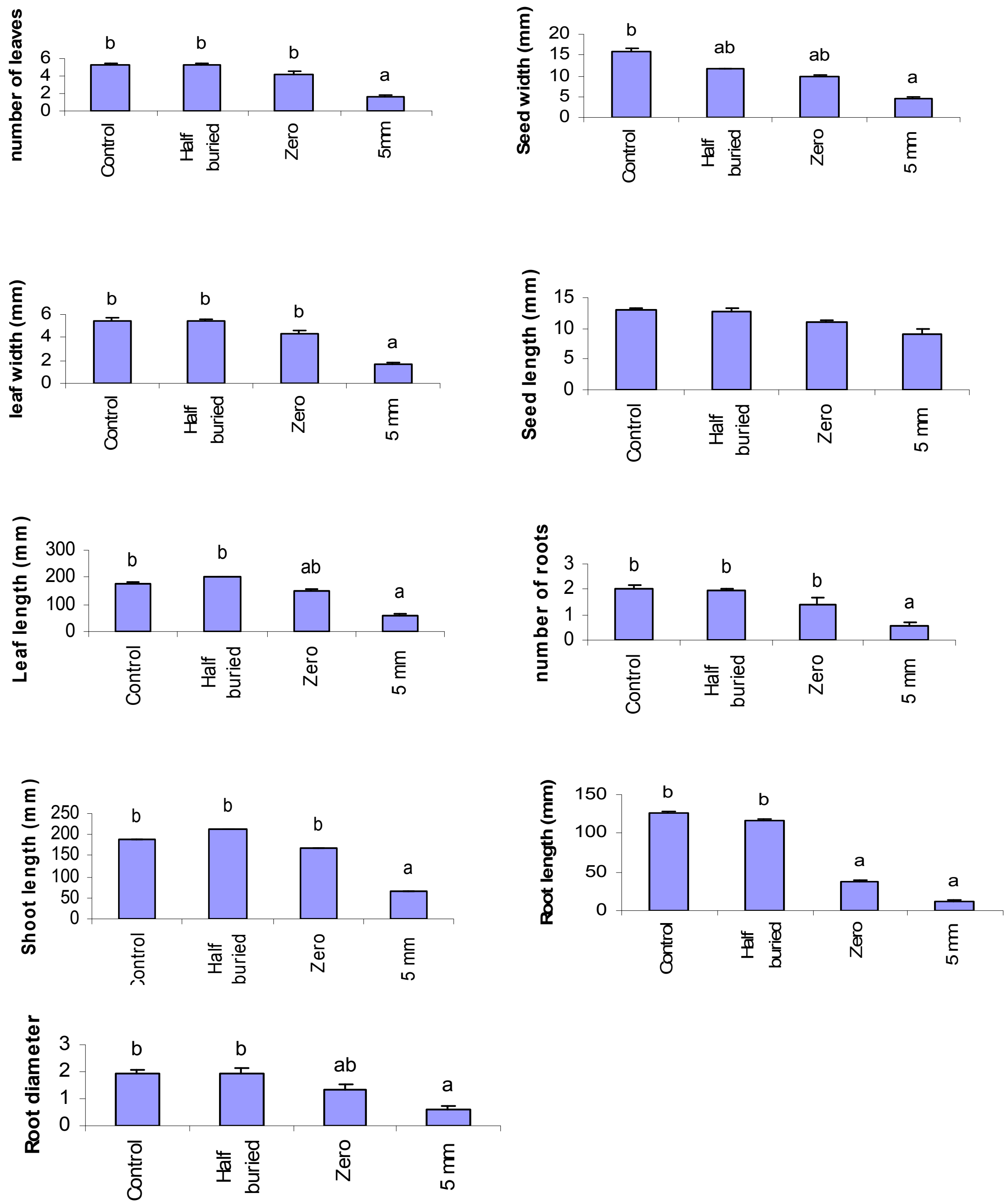

Figure 2. Morphological characteristics of *E. acoroides* seedlings 40 days after the seeds were subjected to different burial depths. Bar graphs with different letters indicate significant difference (Tukey's HSD, $p < 0.05$).

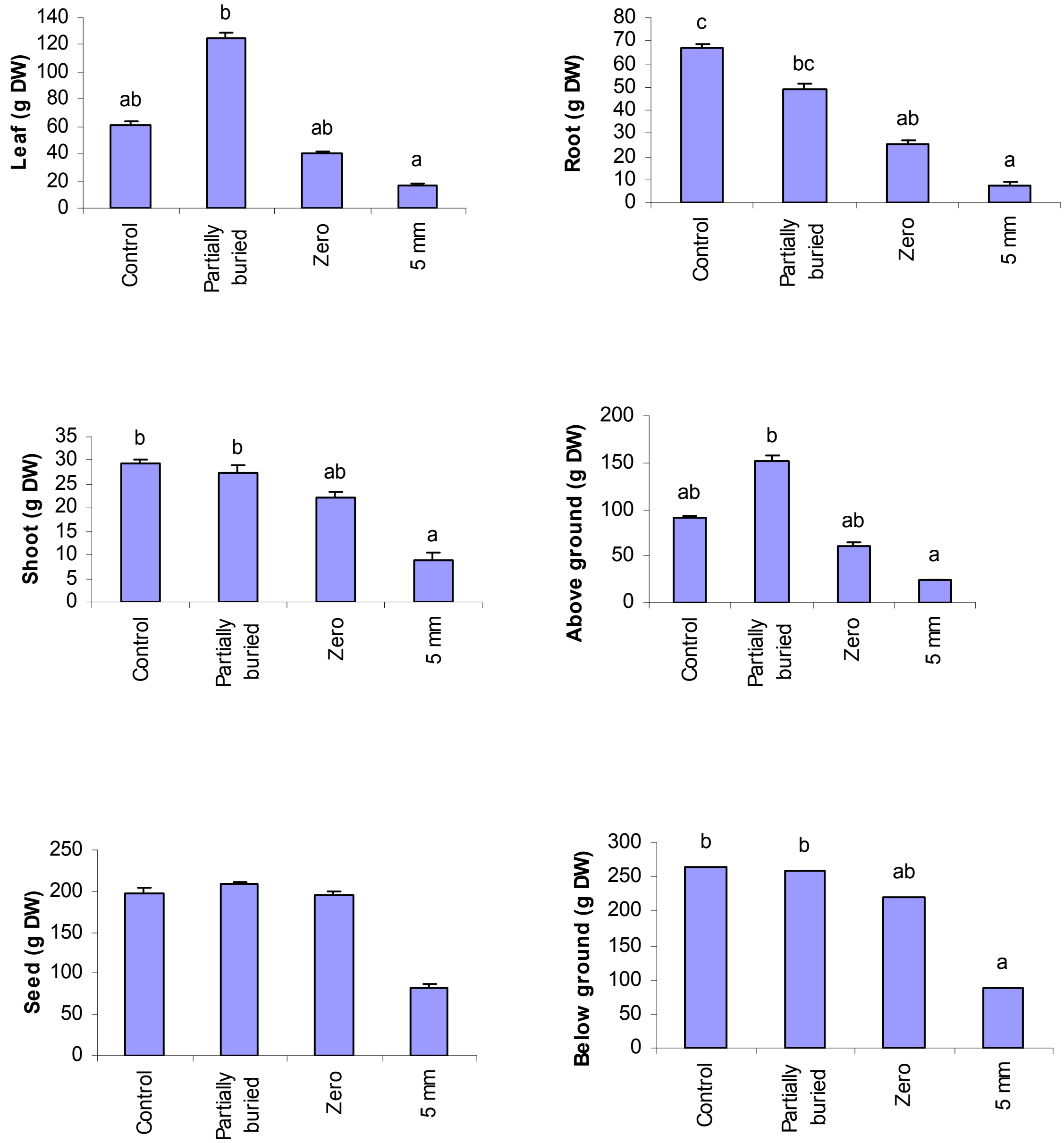

Figure 3. Biomass estimates (g DW) of *E. acoroides* seedlings after 40 days from seeds subject to different burial depths. Bar graphs with different letters indicate significant difference (Tukey HSD, $p < 0.05$).

Table 2. Moisture loss, germination rates, survival, and shoot length of the seedlings, standard errors, from *E. acoroides* seeds desiccated at different periods. Values of one-way ANOVA, written in bold are significantly different ($p < 0.05$). Values with the same superscript letters indicate no significant difference (Tukey's HSD).

Treatments	% Moisture loss	Germination rates	% Seedling survival	Shoot length (mm)
Control	-	100.0	100.0^{b}	196.0^{b}
0.5 hr	$18.88^{a} \pm 9.36$	100.0	100.0^{b}	195.0^{b}
2 hr	$20.76^{a} \pm 9.39$	100.0	93.3^{b}	165.0^{b}
5 hr	$21.85^{a} \pm 4.21$	100.0	86.7^{ab}	165.0^{b}
12 hr	$57.41^{b} \pm 6.38$	96.7	64.8^{a}	75.8^{a}
p values	0.992	0.452	< **0.006**	< **0.001**

DISCUSSION

Failure to germinate and the subsequent deterioration of all seeds at deeper burial depths could be attributed to lack of light. Light availability is a primary factor in determining growth, development and abundance of the seagrass (Uy *et al*., 2001). Deterioration of underwater light, if widespread, could lead to seagrass mortality and is probably linked to reduction in photosynthetic capacity and respiratory requirement (Dennison, 1986; Hemminga and Duarte, 2000). The seeds and seedlings in the 5 mm treatment were also affected by burial depth, thus its lower germination rate. The results of this study parallel the findings of Moore *et al.* (1992) that seeds of *Zostera marina* buried at 5mm under laboratory condition resulted in lower germination success and even mortality of the seedlings.

Shrinking of seeds as a result of desiccation is obviously associated with the loss of water. Desiccation damage may not have been caused only by water loss but also by other factors associated with air exposure. Along with desiccation is the salinity effect, since dehydration tends to affect the water and osmotic potential of plant cells. Exposure to air also causes significant changes in cell temperature (Lobban *et al*., 1985).

Subjecting *E. acoroides* seeds to 12h desiccation must be stressful on the seedling, resulting in stunted growth. The probable consequences of deteriorating quality of the seeds are reduced performance during germination, slower growth rate and development, reduced plant resistance to disease, loss of field emergence, and increased frequency of abnormal seedlings (Copeland, 1976).

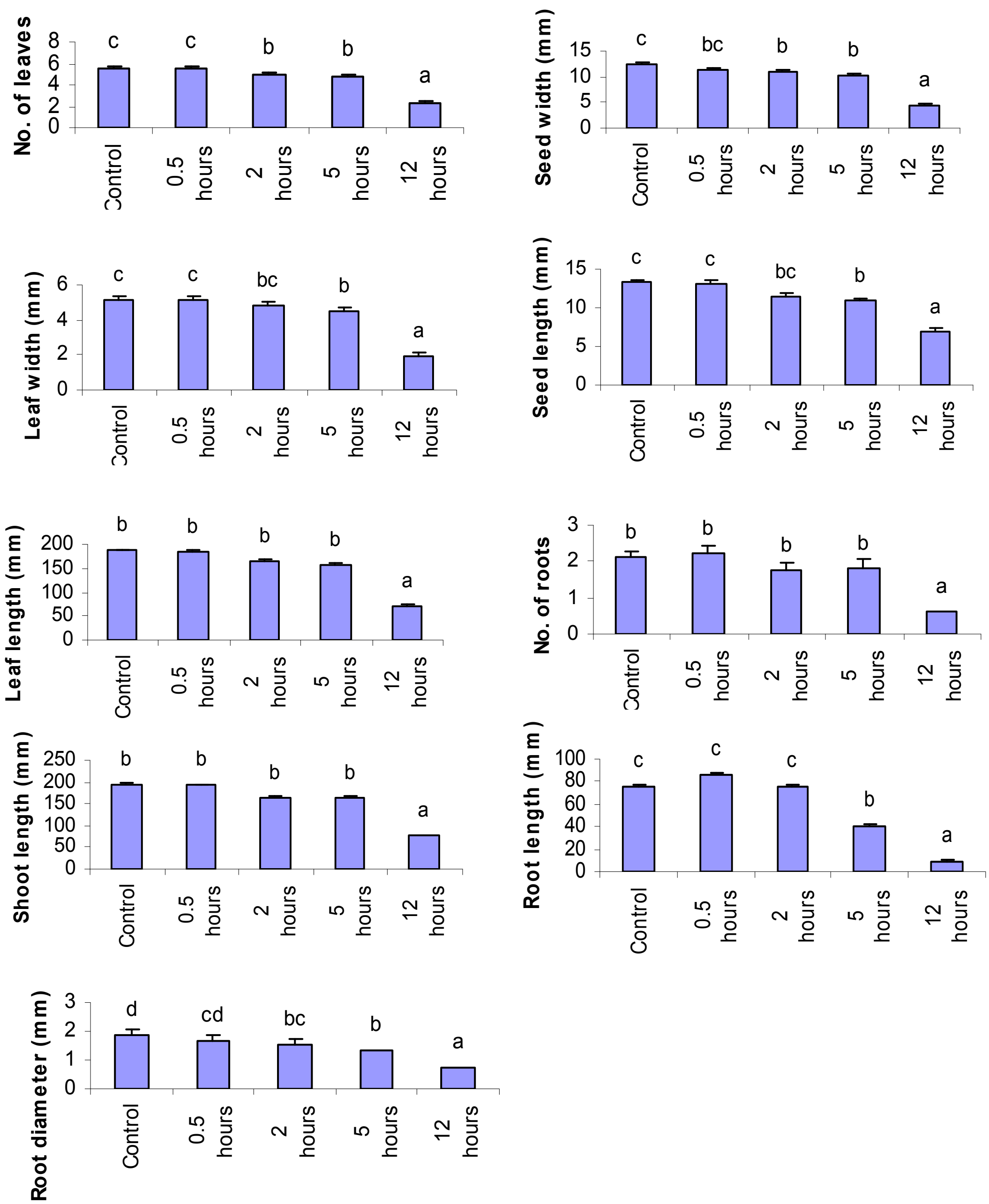

Figure 4. Morphological characteristics of *E. acoroides* seedlings 40 days after seeds were subjected to different desiccation periods. Bar graphs with different letters indicate significant difference (Tukey's HSD, $p < 0.05$).

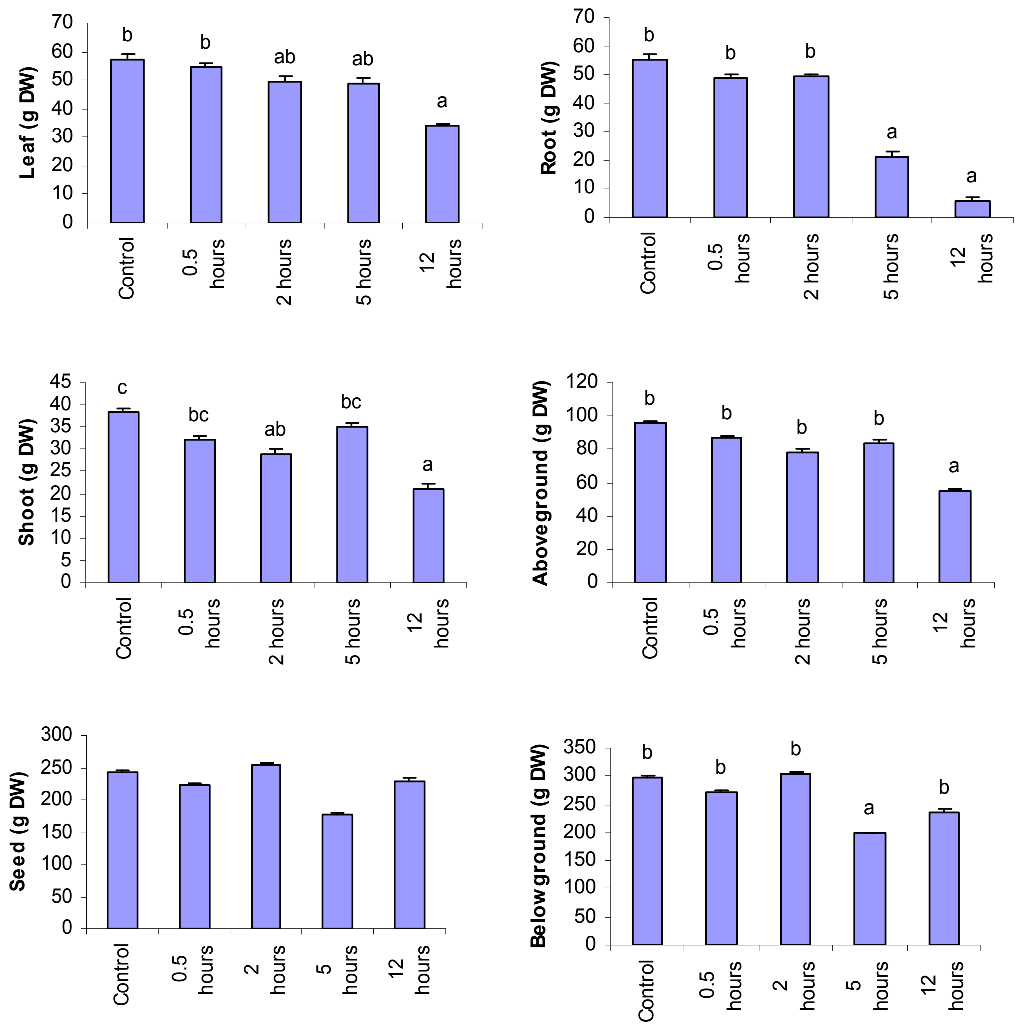

Figure 5. Biomass estimates (gDW) of *E. acoroides* seedlings 40 days after seeds were subjected to different desiccation periods. Bar graphs with different letters indicate significant difference (Tukey's HSD, $p < 0.05$).

CONCLUSIONS AND RECOMMENDATIONS

Results of the study indicated that greater burial depth up to 15mm can cause harmful effects on the germination, seedling survival and development of the seagrass seeds. The present study further showed that deep burial can result in stunted growth and even mass mortality of the seedlings. Although all the seeds subjected to longer desiccation periods (12h) germinated, the seedlings did not fully develop and in fact produced smaller, shorter seedlings. In some cases longer periods of desiccation can result in seedling mortality.

Seeds of *E. acoroides* are best planted either by simply placing them on top of the sediment or partially burying them, which is probably how natural seed germination from seeds directly falling to the sandy bottom occurs. Seeds buried just below the sediment surface can still germinate and develop into a healthy plant. Seeds can be exposed to air or desiccated until 5h with no significant damage to seeds and seedling development. This is an important adaptation of the seagrass which are often subjected to aerial exposure during low tides.

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