

Optimal Salinity, Nitrate and Phosphate Concentrations on Germination and Growth Rate of Eelgrass, *Enhalus Acoroides* (L.F.) Royle

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Abstract: The effects of salinity, nitrate (N) and phosphate (P) concentrations on the germination of *Enhalus acoroides* seeds and growth were studied under laboratory condition. The highest percentage of seed germination was at 20 ppt salinity for all concentrations of N and P. However, there was not different ($P > 0.05$) in the percentage of germinated seed compared with other salinity levels of 25 and 30 ppt at all N and P concentrations. In regard to the growth rate of seedling was found to be effective to the extension of root and lengths. In present study, root and leaf lengths of *E. acoroides* seedling were observed to be significantly declined when the level of salinity and N and P concentrations increased. Hence, alteration in the major nutrient concentration indicates the vulnerability of *E. acoroides* seagrass under culturing laboratory condition, which must be avoidably approached prior to transplantation into the meadow area for conservative management of seagrass resource.

Keywords: *Enhalus acoroides*, Seagrass, Salinity, Nutrient, Germination

I. Introduction

The seagrass population of *Enhalus acoroides*, is one of the most important role of the marine ecological system along coastal and mangrove regions for generating invertebrate and microorganism populations in Thailand [1, 2, 3, 4]. This species have also been widely distributed in many countries in tropical zone of Southeast Asia, such as Philippines, Vietnam, Myanmar, Indonesia, Malaysia, Papua New Guinea, China and Cambodia, due to its ecologically optimal conditions [5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15]. However, the capacity of annual production rate of the seagrass community is often highly depended on the relation of changing seagrass biomass in that time [16].

The several problems have been considerable attention on the reduction of seagrass population included of ability of biomass productivity, distribution and survival or unpredictable phenomenon from natural activities, such as monsoon effect, global warming alteration even through human activities [11, 17, 18, 19]. One of the main problem is that of no seed germination and slow growth rate, which resulted from the light intensity, is reported to be directly associated with consumption of photon gradients via photosynthesis process on the reaction of electron transport chain [20, 21, 22]. Furthermore, there are several factors impacted to the seed germination and seedling growth rate, including salinity, nutrient concentration and type, temperature, light intensity, oxygen concentration and sediment burial [23, 24, 25, 26, 27]. All of them, salinity is a major limiting factor for controlling germination, growth and survival rates of seed and seedling [21, 28]. Nevertheless, the effect of hyper-salinity shock has been well known that resulted from accumulation increased of long-period salt concentration declining of the production capacity in *Thalassia testudinum*, *Halodule wrightii* and *Ruppia maritima* biomass [29, 30].

Recently, many researchers reported that more than 16 species of seagrass have been discovered and well known in the tropical ASEAN regions [4, 18]. Moreover, the suitable concentration of nutrient to the sustainability of seagrass populations was reported in the last decade as a major factor [9, 31, 32]. Although some studies reported about the limitation of nutrient uptake considering on the seagrass growth rate have been successfully focused on other species, available information about the optimal proportion for nutrient uptake has only been focused on the field condition [5, 9]. Hence, approach the appropriate propagation under laboratory condition prior to transplantation to field area is that needed to be investigated for *E. acoroides* resource sustainability. The importance of organic matter limitation of seagrass habitats are considered to be the most essential role involved in biodegradation process as food source for sea cucumbers or crabs. Kilminster et al. [33] outlined that the concentration of N and P nutrient has a correlation with morphological change of root and rhizome compartments to give rise to accumulation of nutrient content (>20 molar) to *H. ovalis* leaf region, which can induce the structural abnormality during trial. Another similar report by Jiang et al. [19] confirmed that growth rate of *T. hemprichii* leaf is sharply depended on sufficient limitation of low light intensity at $25 \mu\text{mol photons m}^{-2}\text{s}^{-1}$ and use of nitrate concentration at $50 \mu\text{mol l}^{-1}$ could gradually damage the property of carbohydrate structure on the leave tissue after 10 days under laboratory condition.

Loss of seagrass beds gives rise to the reduction of ecological and economic values in coastal ecosystems. These events, some country have to legislate, campaign to restore and conserve the seagrass resource [34]. Therefore, the developments the propagating method using seed performed under laboratory condition would be beneficial for sufficient stocking of seagrass seedlings, and obtaining of these data would also be useful for management of the future seagrass resource. However, there is no report about on the nitrate (N) and phosphate (P) concentration under different salinity effected on germination and growth in *E. acoroides*. Hence, the purpose of present study was to investigate the effects of nutrient limitation with use of different N and P concentrations at 0.001, 0.01, 0.1 mg/L and 0.0001, 0.001, 0.01, 0.1 mg/L, respectively, while the salinity level was assigned at 20, 25 and 30 ppt with consideration from the seed germination and seedlings growth of *E. acoroides* seagrass.

II. Materials And Methods

Full ripe of *E. acoroides* seeds, determined from the appearance of brown color of fruit envelopment, were random collected during March-May 2013. The collection was performed in time of the lowest tide at Kung Krabaen Bay (12°35.438'N and 101°53.085'E), Chanthaburi Province, located on the eastern part in the Gulf of Thailand. After sample collection, samples were immediately kept in a zip lock bag, and then placed on crushed ice (~4 °C) in a styrofoam box, prior to transportation to the Department of Aquatic Science laboratory (Burapha University). Container preparation for planting, glass cabinets (117 units) were gained with specially produced instruction. Whereas the soil sample were taken from the same natural source with seed-collected area.

Maturely fresh samples from 585 seeds were used to examine the suitable concentration of nutrient. The study of concentration of N and P in response to growth of leaf and root was immediately performed along with estimation of seed germination after finishing soil preparation. Preparation for salinity study, seawater was adjusted to 20, 25 and 30 ppt salinities (using of previously filtered freshwater for dilution before use). The solutions of NaNO₃ (sodium nitrate; N) and KH₂PO₄ (potassium dihydrogen phosphate; P) were used as the Nitrogen and Phosphorus source respectively. Concentration levels of those were prepared at 0.001, 0.01, 0.1 mg/L as N source and at 0.0001, 0.001, 0.01, 0.1 mg/L as P source. In the control group, salinity was controlled at 30 ppt without addition of NaNO₃ and KH₂PO₄ solutions. The photoperiods were used to allocate of 12 h light and 12 h dark. In all treatments were throughout aerated by electric aerator to generate oxygen consumption during experiment.

Water quality, temperature, dissolved oxygen (DO), and pH was measured as environmental factors. While the seed germination and growth rate was recorded at the end of experiment. For statistical analysis, comparisons of significant difference between testing factors were analyzed using 3-Fixed Factors ANOVA. When the mean difference was detected, DMRT (Duncan's New Multiple Range Test) was also used to compare the difference between groups.

III. Results

3.1 Effect of different concentration level of salinity, N and P to germination of *E. acoroides*

In the environmental factors of temperature, DO and pH parameters was no significant difference in all salinity treatments (20, 25, 30 ppt). Use of low level of salinity concentration (20 ppt) and all N and P concentrations had presented the highest averaged germination (100 %), and was decreased in germination percentage of seed treated in level of salinity in both 25 ppt (at N 0.1 mg/L and P 0.1 mg/L) and 30 ppt (at N of 0.1 mg/L and P 0.01 and 0.1 mg/L) with similar average of 67% germination (P>0.05). Notwithstanding, the reduction in percentage of germinating seed was also observed in treatment concentrating in level of salinity and concentration of N and P supplemented. For statistical analysis, result of correlative linear corresponded in treatment of salinity level and N and P concentration had not effected to the percentage of seed germination (P>0.05).

3.2 Effect of different concentration level of salinity, N and P to growth of *E. acoroides*

The different level of N and P concentrations treated in individual salinity plays an important role for the growth of seedling. An average in leaf and root lengths of *E. acoroides* seedling was significantly different (P<0.05) at all treatments of salinity levels (Fig. 1, 2). At 30 ppt salinity using of N 0.01 mg/L and P of 0.001 mg/L shows maximum the leaf length averaged of 3.9 cm (Fig. 1c), whereas maximum of root length with 3.2 cm average was resulted to 20 ppt at concentration of 0.01 mg/L (N) and 0.0001 mg/L (P) (Fig. 1d). Differently, the minimum value of leaf and root length had also been observed at same salinity (30 ppt) with an average to 0.3 and 0.03 cm, respectively with the same concentration of N and P at 0.1 mg/L (Fig. 1c, 2f). Obviously, the concentration of 0.1 mg/L (N) and 0.1 mg/L (P) reduces forward in seedling growth at salinity level of 25 and 30 ppt with average to leaf length of 0.6, 0.3 cm, and to root length of 0.1, 0.03 cm, respectively, except at 20 ppt the growth of leaf (2.0 cm) and root (1.1 cm) length was significantly higher than that of 25 and 30 ppt. However, average of seedling based on development of leaf and root length was observed at low concentration

to N (0.001 mg/L) and P (0.0001 mg/L), ranging to 2.9-3.3 cm and 2.1-2.5 cm, respectively, which did differ with control group (2.9 cm (leaf length) and 2.3 cm (root length)). An increase of N and P concentrations had the trend to reduce of seedling growth rate observed in all treatments. Above all of this experiment, the application in present treatment of a protocol with concentration of N and P at 0.01 and 0.001 mg/L, respectively dissolved in level of salinity at 30 ppt seawater was determined to be the most appropriate conditions, determining from leaf and root lengths of seedling differentiated during experiment see in Fig. 1c, 2f.

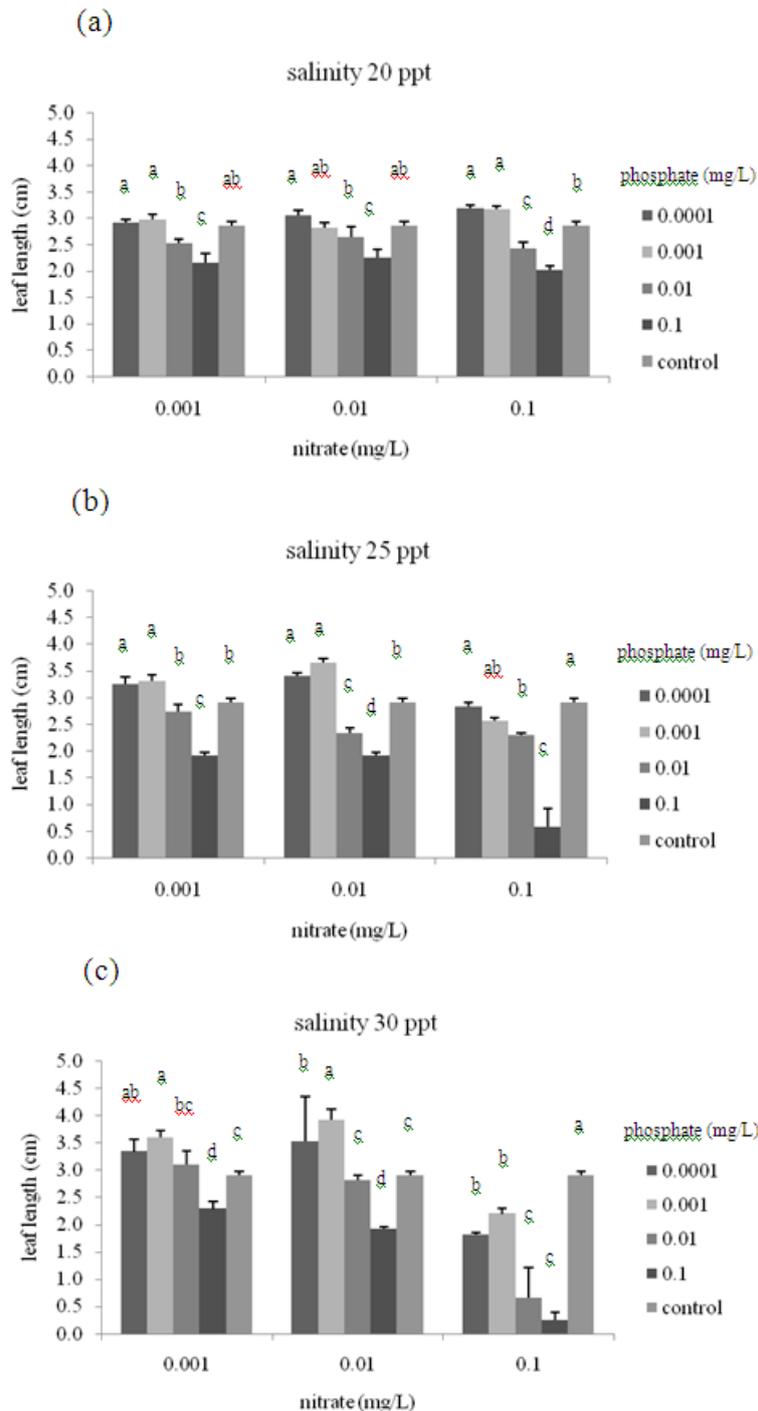


Figure 1. The leaf length (cm) of *E. acoroides* seedlings with different salinity, N and P concentrations, the leaf length with regard to treatment of salinity level at (a) 20 ppt; (b) at 25 ppt; (c) at 30 ppt (bars represent standard errors).

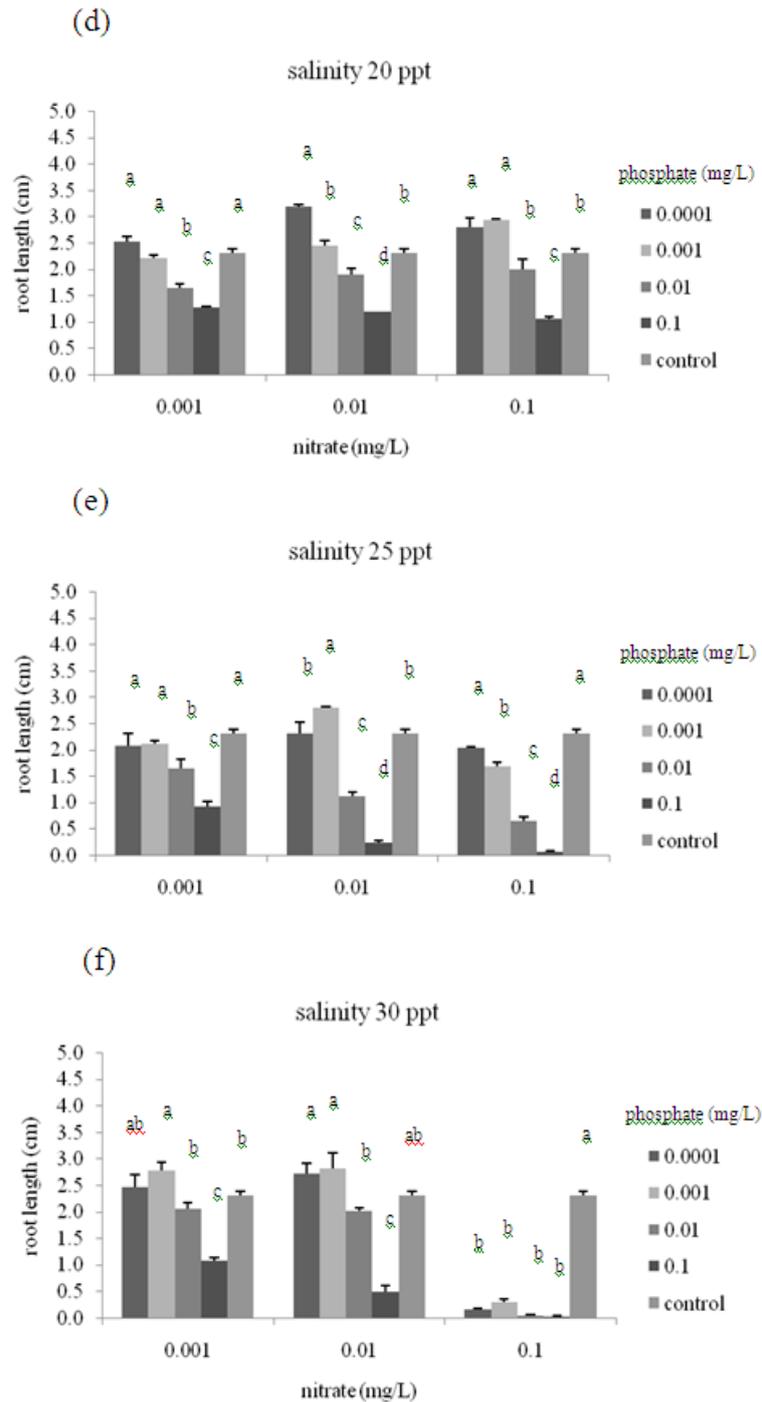


Figure 2. The root length (cm) of *E. acoroides* seedlings with different salinity, N and P concentrations, the root length with regard to treatment of salinity level at (d) at 20 ppt; (e) at 25 ppt; (f) at 30 ppt (bars represent standard errors).

IV. Discussion

4.1 Effect of different concentration level of salinity, N and P to germination of *E. acoroides*

E. acoroides can better tolerate the change of salinity level than other species (*H. uninervis*, *T. hemprichii*, *Halophila ovalis*, *Cymodocea rotundata* and *Syringodium isoetifolium*) [35, 36]. The present study, level of different salinities as well as N and P concentrations used in all treatments did not affect on percentage of seed germination. This is possible that the storage of essential materials inside the seed to generate germination has enough to grow prior to development to be the seedlings via photosynthesis pathway [37, 38, 39]. Empirically, germination of *E. acoroides* seeds was able to occur in all conditions (in this study), but it could greater germinate at low salinity level. Similarly, Sim et al. [40] found that the germination response was

successfully reported using the salinity level between 15-30 ppt in *R. megacarpa* seagrass under wetland system. Fernández-Torquemada and Sánchez-Lizaso [28] also observed that the highest percentage of germination of *Posidonia oceanica* seeds (90%) was occurred, controlling salinity at 37 ppt, while use the level of 40-49 ppt salinity provides lower germination average. It is may directly be due to the level of higher salinity could induce with the decrease in amount of germinated seeds before differentiation in other phases and was able to provide the longer period for beginning germination observed in *Z. capricorni*, *R. tuberosa*, *R. megacarpa* and *R. maritima* species [27, 30, 41].

Receiving of present data with optimal protocol could confirm the success of propagation program using seed obtained natural resource with culture under laboratory condition. Utilization of these stocking would reduce the problem that is being occurred with lack of seagrass resource as primary producer. Although presence of seed germination rate in all treatments had yielded with high percentage, one of the most important barriers for inhibiting seed germination is the fungal infection found among study, presenting the characteristic of light white color was dispersed around the tip of the seed surface area within 48 hrs after planting. This is possible that the setting location for planting was operated under condition of wet laboratory system, thus risking for adhesion of spore to that area of seed surface could be possible simply, which inside the seed contains of biomaterial compound as food reserve for theirs growth. To date, research report about infection of fungi cluster as environmental pathogen associated with delaying of seed germination in any species of seagrass population has been not approached. Due to this problem is one of another important factor for failing seed germination.

4.2 Effect of different concentration level of salinity, N and P to growth of *E. acoroides*

There was the report about the ability of tolerance to fluctuated salinity of seagrass in seedling phase have more the sensitivity of response to stress than adult phase [28, 42]. Presently, effects of *E. acoroides* seedling treated in level of 20, 25, or 30 ppt salinities was significantly different in average of growth rate ($P < 0.05$) within a first week after cotyledon germination. Koch et al. [29] who argued that the use of hyper-salinity level studied in *T. testudinum* adult phase had well been tolerant to the acclimation in salinity fluctuated between 36-70 ppt using restricted circulation system. In contrast, percentage of moderate survival ($>50\%$) in *R. maritima* seagrass specioes with providing of salinity up to 45 ppt, and supplementation for salinity concentration was over 50 ppt lead to complete mortality of *T. testudinum* [24, 30]. Presently obtainable information, growth rate for seedling phase in all treatments on each salinity level were different comparing with control segment. In fact that the response of the growth rate of *E. acoroides* leaves to the changed concentration in each salinity levels have the relation with osmoregulation to acclimate between intracellular and extracellular region. However, previous research indicated that the salinity fluctuation has an effect on osmotic pressure within *T. testudinum* cells that could suddenly adapt to the changes in level of fluctuated salinity [24], especially the alteration of sudden salinity level at the area of the coastal or estuarine regions was also reported that an immediate alteration of concentration level of salinity would induce an abnormality or mortality events of *Z. marina*, *Z. noltii*, *Z. capricorni*, *Z. capensis* and *C. nodosa* seagrass [29, 43]. Similar achievement studied by Kahn and Durako [24] who examined the response of seedling development of *T. testudinum* seagrass to the condition of environmental stress on use of enrichment of water chemical compositions (hypo-hyper-salinity conditions (ranged 0-70 ppt). However, present result showed that the level of salinity at 30 and 40 ppt provided the greatest growth rate. this is presumable that the metabolic rate of photosynthetic reaction that is relative with electron transport chain in a process of tricarboxylic acid (TCA) cycle hardly increased in this period (30-40 ppt) when compared with other ranges.

Notwithstanding, an increase of nutrient concentration in this study did supplement the growth rate of *E. acoroides* seedlings. There was the report with content of N and P accumulation in leaf compartment that have more double concentration than root and rhizome compartments. Kilminster et al. [33] have suggested that a large number of plant organisms need the level of high P concentration to store in *H. ovalis* leaf for growth and also appeared the enrichment of N and P accumulation source, absorbed of N and P concentration to 20.4% and 21.9%, respectively [44]. Although in this study, it has not been estimated the absorption and accumulation of N and P nutrient in leaf, the growth rate with leaf length increased since the first germination (3-4 days) to late experiment (1 month) could confirm preliminary that the change of leaf length was essential to be dependent on nutrient concentration dissolved in sea water in *E. acoroides*, averaged the lowest leaf length to 0.3 cm in treatment of using 30 ppt salinity at a concentration of N to 0.1 mg/L and P to 0.1 mg/L (see in Fig. 1c).

Over accumulation of nutrient content of N and P is another important factor that could also cause with proliferation in number of macro-microalgae, phytoplankton or epiphytes [32, 45]. This is often occurred under laboratory condition with the presence in color of water changed from transparent to light green. It is likely the cluster of plate-algae floating on water surface area after 5 days planting (visual observation). This may be due to obtaining of light intensity is enough to a bottom area to proliferate the algae cluster via photosynthesis cycle

[19, 32]. The effect of inhibition in development of *Z. marina* seedling was also concluded about an efficiency of photosynthesis cycle that is significantly decreased when the concentration of elements supplemented as laboratory condition [31]. Similarly, the presence with ability of photosynthesis reduced during experiment which can bring to significant decreasing of growth and distribution rates in *P. oceanica* seagrass due to limiting light transmission at the area of bottom pond [46]. In consequence, there was the decrease in consumption of oxygen content, which was considered to be associated with respiration system. Condition of low light intensity, the respiratory demand was reduced, especially in the shoot compartment, also found in *P. sinuosa* seagrass with declining of photosynthesis rate have direct effect on size of smaller leaf [46, 47]. Additionally, Niu et al. [26] has suggested about the effects of respiration rate related to the oxidative phosphorylation for synthesis the high energy substance (ATP) from stored carbohydrate source. For projection of the first cotyledon within the first week period after seed burial was successful using a treatment of 30 ppt seawater at a concentration of N to 0.01 mg/L and P to 0.001 mg/L (Fig. 1c). This available information may be due to the storage of energy content had been enough to accelerate the germination of cotyledon compartment was better than other treatments. Notwithstanding, the important roles for first germination factor of cotyledon projection was previously known, included of fat and protein contents to receive the monosaccharide from gluconeogenesis pathways as a catabolism, and to synthesize the fatty and amino acids as anabolism pathways described by Niu et al. [26].

V. Conclusion

In conclusion, presents results demonstrated that an altering salinity among 20-30 ppt of N and P concentrations did not affect to the germination condition of *E. acoroides* ($P > 0.05$). In contrast, the level of all salinity embedded in N and P concentration in all treatments closely influenced the growth rate. Obviously, with environmental factors, of temperature, DO and pH fluctuations throughout the experiment period were not different with control treatments. However, application with achievement of these results for utilizing seedling could only be practical under laboratory condition, but the response for adaptation in each seedling after transplantation to environmental condition may be failed if any environmental factors are not suitably promoted with and without unpredictable phenomenon for growth development to the adult *E. acoroides* seagrass that is unclear.

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