



EFFECTS OF SALT STRESS ON PLANT GROWTH AND BIOMASS ALLOCATION IN SOME WETLAND GRASS SPECIES IN THE MEKONG DELTA

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Abstract. Salt stress causes serious damage to many cellular and physiological processes that leads to yield reduction. The study induced salt stress using Hoagland solution added NaCl to evaluate its effects on plant growth and biomass allocation of some wetland grass species in order to identify salt-tolerant species for replacing and/or supplementing rice/grass in rice-shrimp model and salt-affected area in the Mekong Delta. The study also seeks to evaluate the response of leaf chlorophyll (SPAD unit) and proline content in salt-treated plants to varying application of salinity. *Typha orientalis*, *Lepironia articulata*, *Eleocharis dulcis* and *Scirpus littoralis* were studied in hydroponics condition with four levels of NaCl of 5, 10, 15, 20 ‰ and the control treatment (without adding NaCl). The study was performed with completely randomized factorial design having in triplicate. The experiment was carried out at the College of Environment and Natural Resources of Can Tho University from July to November 2018. Morphological toxic symptoms and growth inhibition were clearly observed in salt-treated treatments. Among the four studied species, *T. orientalis* produced the highest dry shoot biomass (15.5 g DW/plant), while *E. dulcis* had the lowest value (2.8 g DW/plant). However, only *T. orientalis* showed significantly decreased in biomass as salinity increased with 9.3 and 4.6 times lower of fresh and dry biomass in plants grown at the salinity level of 20 ‰ compared to those grown in the control treatment. The other three plant species did not affect by salinity levels. The results indicated that *S. littoralis*, *L. articulata* and *E. dulcis* could tolerate at high salinity of 20 ‰ (eq. to the EC value in the nutrient solution of 38.0 dS/m) and could be potential candidate to grow in the rice-shrimp model or in the salt-affected soils.

Keywords: growth, salt stress, salt-tolerance, wetland plant.

Classification numbers: 3.1.1, 3.5.1, 3.7.2.

1. INTRODUCTION

The Mekong River Delta (MRD) in Viet Nam is the largest agriculture and aquaculture production region of the nation. However, the MD has been described as one of three 'extreme' world hot-spots in terms of probable population displacement resulting from rising sea level by the Intergovernmental Panel on Climate Change (IPCC) [1]. According to Duc *et al.* the MD has 0.88 mil ha of saline soil accounted for 36.6 % of the total area which was in the second soil type after alluvium soil [2]. Salt water intrusion into the mainland causes a shortage of freshwater for irrigation [3] that is the most concerned problem in the coastal areas of the MD [4]. Salt stress causes serious damage to many cellular and physiological processes, including photosynthesis, nutrient uptake, water absorption, plant growth, and cellular metabolism, all of which lead to yield reduction [5]. Maximum tolerable salinity with no yield penalty comparing to respective control treatment and the percentage reduction in yield per unit of salinity greater than the limit are two key criteria for salt-tolerant crops [6]. In addition, the survival and growth rate, difference of percent biomass yield between saline and control conditions over experimental time-course are typical measurements in salt tolerant experiments. Moreover, plants under salinity stress produce more proline but reduce in chlorophyll in leaves [7, 8] which are considered as indicator for plant response to salinity stress and those plants were classified as salt-sensitive species. In the present study, Sedge (*Scirpus littoralis* Schrab), Cattail (*Typha orientalis* C. Presl), Bulrushes (*Lepironia articulata* (Retz.) Domin) and Water chestnut (*Eleocharis dulcis* (Burm.f.) Trin. ex Hensch.) were chosen to study salt-stress. In fact, the four plants are cultivated in the paddy field and/or in the fish and/or shrimp ponds in the MD for human food and providing habitat for fish and shrimp growth [9]. Among them, *S. littoralis* had higher salt-tolerant capacity than that of *T. orientalis* with salinity NaCl levels of 20 - 30 ‰, while *T. orientalis* had salinity tolerant threshold at 10 ‰ [9], thus *S. littoralis* was considered as the reference plant in the present study. However, there are limited information on salt-tolerant capacity of *L. articulata* and *E. dulcis*. Consequently, the research was conducted to find out the potential salt tolerant species among four wetland plants to grow/replace rice in the rice-shrimp model to adapt with climate change.

2. MATERIALS AND METHODS

2.1. Experimental setup

A completely randomized design consisting of four wetland plant species (*Scirpus littoralis*, *Typha orientalis*, *Lepironia articulata* and *Eleocharis dulcis*) and five concentrations of NaCl solutions (0, 5, 10, 15, 20 ‰) was arranged in the net house in triplicate. The experiment was performed at the College of Environment and Natural Resources of Can Tho University, Viet Nam (10.03° N latitude and 105.76° E longitude) from July to November 2018.

2.2. Plant materials and salinization

Rhizomes and young plants of the four studied species were collected from the fields in Phuoc Long district, Bac Lieu province and Phu My district, Kien Giang province on July 2018. Two individual young and similar size plants for each species were placed in each 9.0 L container. The height of initial young plants of *Typha orientalis*, *Lepironia articulata*, *Eleocharis dulcis* and *Scirpus littoralis* were 100 - 120 cm; 60 - 90 cm; 50 - 80 and 80 - 110 cm. Eight point five liters of an incremental concentration from a quarter-strength to half-strength

Hoagland's solution [10] was used for the first three days and the next four days, respectively. The full-strength Hoagland's solution was applied as nutrient supplement from the second week to the experimental completion. On the day 14th after transplant, salt was added to hydroponic solution. A weekly and stepwise increment of 5 ‰ NaCl until reaching the salt concentration of 20 ‰ was applied for Hoagland's solution [9]. A refractometer (Alla, France) was used for measurement of salinity. In addition, nutrient solutions were weekly renewed to make sure sufficient nutrients for plant growth. The control treatment was 0 ‰ NaCl. The electric conductivity (EC) values in the prepared Hoagland solution of the treatments 0, 5, 10, 15 and 20 ‰ were 2.2, 6.6, 19.4, 29.5 and 38 dS/m, respectively.

2.3. Plant growth and biomass allocation determination

The height of shoots was measured weekly at the root base to the highest leaves, while the length of root (from the root base to the longest root) was measured at the beginning and the end of the study with a ruler [9]. A total of 49 days was the whole study period in which 14 days was without salinization and 35 days was salinization. At harvest, the plants were carefully removed from the growth solution and the roots were washed in distilled water before separated into roots and shoots. The shoots term we used in this study was comprised of stems and leaves. The root and shoot fractions were weighed for fresh biomass and dried at 60 °C until constant weight to determine for dry weight [9].

2.4. Leaf chlorophyll and proline content

In addition, before harvest plants we measured chlorophyll in the third leaf from top using SPAD Konica Minolta meter (Model SPAD-502 Plus, Tokyo, Japan). SPAD reflects chlorophyll in the leaf [11]. After measure SPAD, the leaves at the third, fourth and the fifth position [12, 13] were collected to determine proline content [14].

2.5 Statistical analysis

Analysis of variance (ANOVA) and the Tukey Honestly Significant Differences (HSD) post hoc test were performed using Statgraphics Centurion XV (StatPoint, Inc., Warrenton, VA, USA). The assumptions of normality, homogeneity of variance and logarithmically transformed (if necessary) were fulfilled prior to ANOVA analysis. Two-way ANOVA (species x salinity levels) of plant growth, biomass and mineral content in plant fractions was performed to examine differences of means. One-way ANOVA was used to determine salinity effects within species and species effects within salinity. Significant differences among plant species and salinity levels ($\alpha = 0.05$) were detected using Tukey HSD.

3. RESULTS AND DISCUSSION

3.1. Salt-stress effects on plant growth

After 35 days salinization, salinity did not affect survival rate of the four plant species (Fig. 1) with no mortality was observed in all salinity levels ($p > 0.05$; Fig. 2A). However, among the four studied plants, salt-stress symptom of leaf rolling and wilting starting at the salinity levels of 10 ‰ occurred strongly in *T. orientalis* (Fig. 1A). In the other study, Trang *et al.* [9] reported that *T. orientalis* had 65 % of plant survived at the salinity levels of 10 and 15 ‰, while only

35 % of *S. littoralis* plant survived at the salinity levels of 20 and 30 ‰. It could be explained that *T. orientalis* in the present study exposed to salinity stress with shorter duration in total of 35 days, whereas in Trang *et al.* [9] *T. orientalis* was salinized in 38 days. Typha species are well known to be moderately salt tolerant. Crain *et al.* [15] reported that narrowleaf cattail (*T. angustifolia*) had a reduction of 22% biomass as grown at 20 - 30 ‰ salinity as compared to that at 0 ‰ level while Konisky and Burdick [16] documented only 50 % survival of transplants at a salinity of 10 ‰ in the field experiment. *S. littoralis* had 100 % plant survived which was similarly reported by Trang *et al.* [9]. *L. articulata* and *E. dulcis* are well known as acid sulfate soil indicators; however, they also were recognized as salt-tolerant potential candidate at the high salinity level of 20 ‰ with 100 % plant survived (Fig. 1B, 1C & 2A) in the present study. In addition, there were no reduction in number of new shoots, height of shoot, and root length of the four plant species ($p > 0.05$; Figs. 2B, 2C & 2D), except for shoot height of *T. orientalis* ($p < 0.05$; Fig. 2C). Doan *et al.* [17] reported that *T. orientalis* could survive in the constructed wetland system treated recirculated intensive whiteleg shrimp tank culture at salinity level of 10‰. In the other study, Thanh *et al.* [18] also recorded from the shrimp farmers' experiences about salinity tolerant capacity of *T. orientalis* was 5 - 10 ‰. Therefore, only consider to grow *T. orientalis* in the rice-shrimp model during the rainy season or in the location where salinity was at 5 - 10 ‰ (eq. to the EC value of 6.6 - 19.4 dS/m in the nutrient solution).

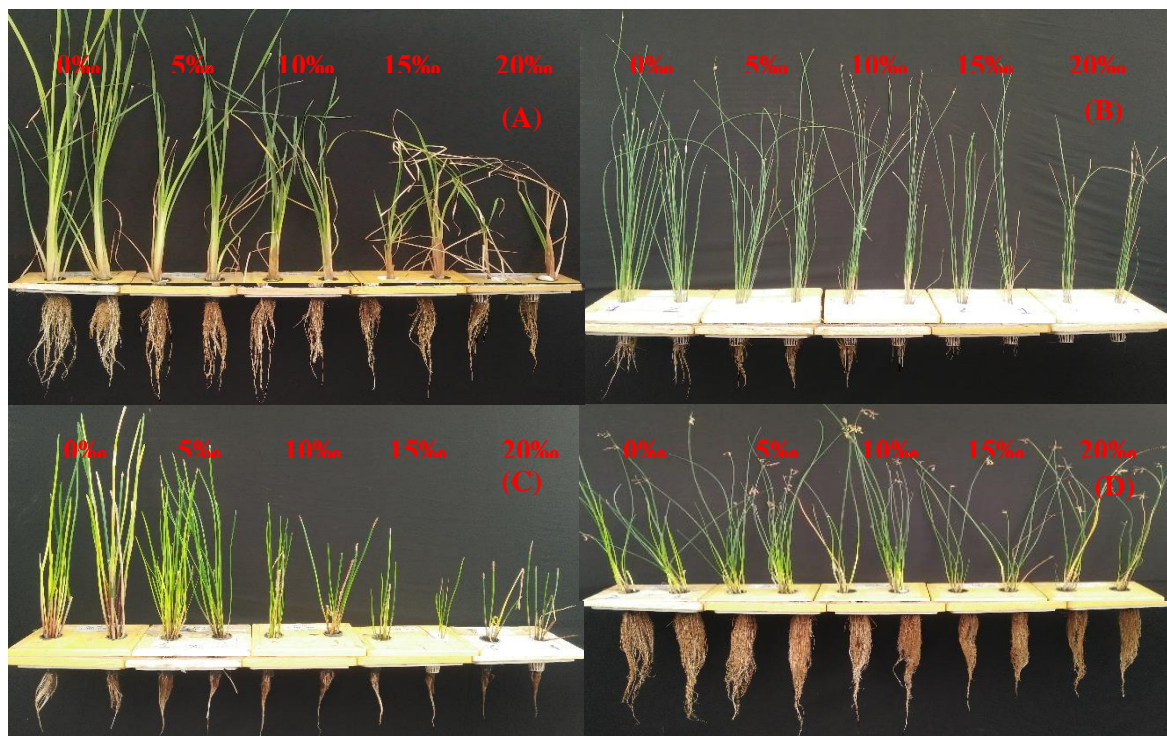


Figure 1. Phenotypes of *Typha orientalis* (A), *Lepironia articulata* (B), *Eleocharis dulcis* (C) and *Scirpus littoralis* (D) grown at different salt concentrations.

3.2. Salt-stress effects on plant biomass allocation

Similar to height of plants and length of roots, salinity did not affect fresh weight (FW) and dry weight (DW) of the shoot and the root systems of the four studied plants ($p > 0.05$; Table 1), except for fresh weight and dry weight of the shoot and the root of *T. orientalis* ($p < 0.05$; Table 1). The results showed that all the four studied plants had the same trend in reduction of FW and DW of the shoot and the root systems as salinity concentrations increased in the nutrient solution (Table 1).

Table 1. shoot and root fresh weight (FW) and dry weight (DW) of the plants treated with salinity levels of 5, 10, 15, 20 ‰ and the change (%) as compared to the plants in the control treatment (0 ‰).

Species	Salinity levels	Shoot FW (g/plant)	Change (%)	Root FW (g/plant)	Change (%)	Shoot DW (g/plant)	Change (%)	Root DW (g/plant)	Change (%)
<i>Typha orientalis</i>	0	166.1 ^a	100	15.4	100	15.5 ^a	100	1.5	100
	5	70.5 ^{ab}	42.4	18.0	116.9	7.2 ^{ab}	46.4	1.5	100.6
	10	46.7 ^b	28.1	14.1	91.7	5.0 ^b	32.5	1.1	72.4
	15	27.3 ^{bc}	16.4	13.5	88.0	4.4 ^b	28.1	1.2	80.4
	20	17.8 ^c	10.7	11.6	75.0	3.4 ^b	21.8	0.8	54.3
P-values		0.0002^{***}		0.669 ^{ns}		0.0018^{**}		0.272 ^{ns}	
<i>Lepironia articulate</i>	0	12.7	100	8.6	100	3.8	100	1.8	100
	5	10.9	85.4	6.6	76.3	3.3	87.8	1.0	58.4
	10	8.2	64.6	4.7	55.0	2.6	69.9	0.8	44.3
	15	8.8	69.1	4.8	56.3	3.3	87.1	0.9	49.2
	20	6.3	49.7	4.2	48.2	2.6	67.4	0.6	33.0
P-values		0.104 ^{ns}		0.324 ^{ns}		0.199 ^{ns}		0.093 ^{ns}	
<i>Eleocharis dulcis</i>	0	33.6	100	9.0	100	2.8	100	0.6	100
	5	23.4	69.6	13.1	144.7	1.1	39.5	1.7	285.9
	10	14.0	41.6	5.7	63.4	1.3	46.2	0.5	78.5
	15	6.5	19.5	4.9	53.9	0.7	24.5	0.6	99.6
	20	5.4	16.1	5.6	61.9	0.6	20.7	0.7	123.5
P-values		0.117 ^{ns}		0.663 ^{ns}		0.544 ^{ns}		0.712 ^{ns}	
<i>Scirpus littoralis</i>	0	43.6	100	9.9	100	5.1	100	1.6	100
	5	47.2	108.2	21.0	212.8	5.5	107.2	3.5	216.2
	10	31.0	71.2	17.1	173.3	4.9	95.7	2.1	132.2
	15	21.1	48.3	18.2	184.2	2.8	53.9	2.4	147.8
	20	25.0	57.4	18.6	188.8	3.6	69.5	2.7	170.8
P-values		0.212 ^{ns}		0.346 ^{ns}		0.187 ^{ns}		0.523 ^{ns}	

Notes: Values are the means of 3 replicates. Different letter ^{a,b,c} within the column indicates significant difference between salinity levels within plant species. **: $p < 0.01$; ***: $p < 0.001$; ^{ns}: $p > 0.05$.

However, there was no significant difference in FW and DW between the salt-treated plants and the control plants (without adding NaCl in the growth solution). The shoot FW and

DW of *T. orientalis* (70.5 and 7.2 g/plant) in the salinity concentration of 5 ‰ (eq. to the EC value of 6.6 dS/m) were reduced 54 – 57 % compared to the plant grown in the control treatment of 0 ‰ (Table 1); however, there was no significant difference was detected ($p > 0.05$; Table 1). The salinity concentration increased at the level of 10 ‰ (eq. to the EC value of 19.4 dS/m) the shoot FW and DW of *T. orientalis* (46.7 and 5.0 g/plant), which were significant lower than those grown in the control treatment ($p < 0.05$; Table 1). The results showed that high NaCl levels only inhibited shoot FW and DW of *T. orientalis* and salinity level of 10 ‰ was the threshold NaCl for *T. orientalis*.

In sum, *S. littoralis*, *L. articulata* and *E. dulcis* could tolerate the high salinity concentration of 20 ‰ (eq. to the EC value of 38.0 dS/m). Our previous study on native and/or cultivated plant species showed that the presence of *S. littoralis* in brackish shrimp pond helped to improve the pond ecosystem and to enrich sources of organic feed materials and density of natural food in the pond [18]. Importantly, *S. littoralis*, a high salt-tolerant candidate, appeared to be the plant of choice in the semi-intensive farming with one shrimp crop and one sedge crop. In addition, they also said that *S. littoralis* tolerated at the salinity concentration of 12 - 15 ‰, while *E. dulcis* could tolerate at the higher salinity concentration of 16 - 20 ‰ [18].

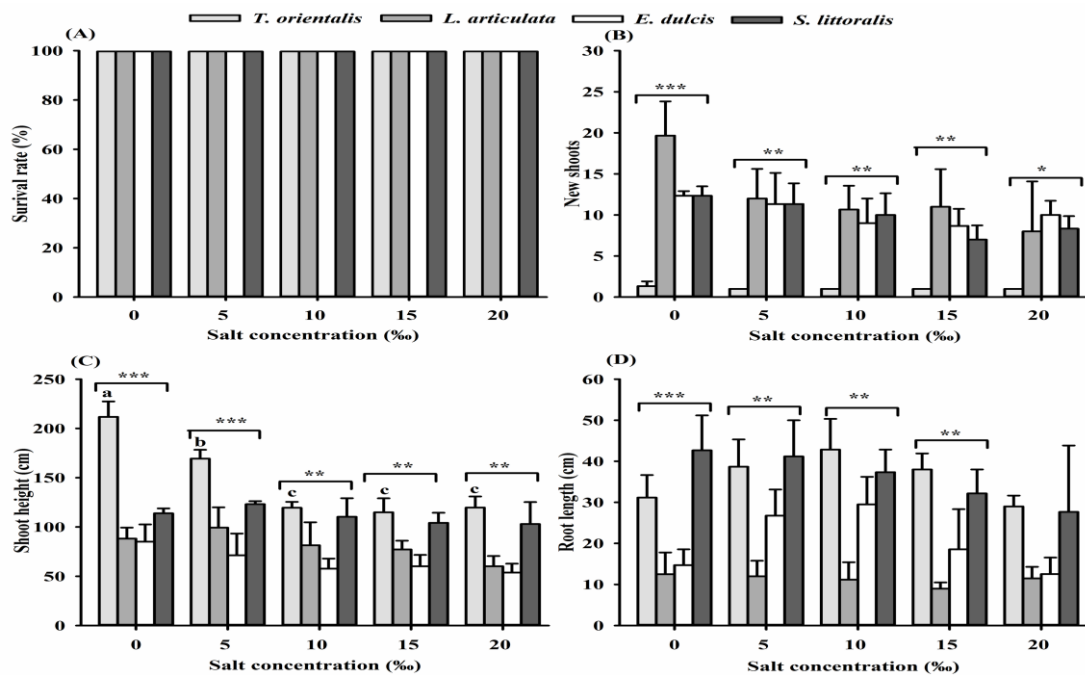


Figure 2. Survival rate, % (A); number of new shoot (B), shoot height (C) and root length (D) of *Typha orientalis*, *Lepironia articulata*, *Eleocharis dulcis* and *Scirpus littoralis* grown at different salt concentrations. Values are the means of 3 replicates \pm S.D. Asterisk (*) indicates significant difference between four plant species within salinity level. Different letter ^{a,b,c} indicates significant difference between salinity levels within plant species.

3.3. Leaf proline and chlorophyll content

The content of proline and chlorophyll in the leaf of *T. orientalis*, *L. articulata*, *E. dulcis* and *S. littoralis* grown at the different salinity concentrations was presented in the Fig. 3. The

results showed that salinity levels affected the leaf proline content of the four studied plants ($p < 0.05$; Fig. 3); proline was accumulated in the leaf higher at the higher level of NaCl. The leaf proline content of *T. orientalis*, *L. articulata*, *E. dulcis* and *S. littoralis* grown in the control treatment were 0.69, 3.73, 0.68 and 0.48 $\mu\text{mol/g}$ FW, respectively, and it was increased at 6.13, 13.99, 2.42 and 15.76 $\mu\text{mol/g}$ FW of the respective plant species grown in the salinity level of 20‰ (equivalent to an increase of 8.9, 3.7, 3.6 and 32.9 times). According to Chiang and Dandekar [19] and Turan *et al.* [20] proline accumulation is believed to play adaptive roles in plant stress tolerance. *S. littoralis* had the highest amount of proline accumulated in the leaf in the salinity level of 20‰ among the four studied plants. Trang *et al.* [9] found that *S. littoralis* accumulated the lower concentration of Na^+ and Cl^- in the shoots and the roots compared to *T. orientalis* at all NaCl levels which can be associated a higher salt tolerance capacity of *S. littoralis*.

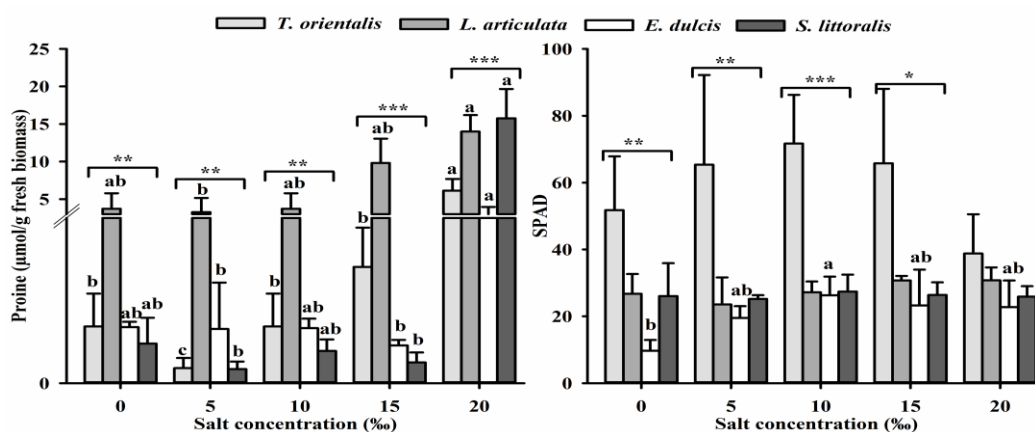


Figure 3. Leaf proline ($\mu\text{mol/gFW}$) and chlorophyll content (SPAD) of *T. orientalis*, *L. articulata*, *E. dulcis* and *S. littoralis* grown at different salt concentrations. Values are the means of 3 replicates \pm S.D. Asterisk (*) indicates significant difference between four plant species within salinity level. Different letter ^{a,b,c} indicates significant difference between salinity levels within plant species.

In contrast to proline content accumulation, total leaf chlorophyll content was reduced as the salinity levels increased; however, only *E. dulcis* had significant increase in total chlorophyll content in the plant grown in the treatment of 10‰ compared to those grown in the control treatment ($p < 0.05$; Fig. 3). Many scientists reported that the total chlorophyll concentration of plant leaves was reduced by increased level of NaCl treatment [20, 21].

4. CONCLUSION

After 35 days salinization, salinity did not affect survival rate of the four plant species with 100% survival rate was observed in all salinity levels. However, only *T. orientalis* showed the lower salt-tolerant capacity among the four studied species with a reduction of the shoot FW and DW in the salinity levels of 5‰ and 10‰, which were 54 – 57% and 68 – 72% compared to the control plant (0‰). The leaf proline content of *T. orientalis*, *L. articulata*, *E. dulcis* and *S. littoralis* grown in the control treatment were 0.69, 3.73, 0.68 and 0.48 $\mu\text{mol/g}$ FW, respectively, and it was increased 8.9, 3.7, 3.6 and 32.9 times in the respective plant species grown in the salinity level of 20‰. Taken together, *S. littoralis*, *L. articulata* and *E. dulcis* could tolerate the

high salinity concentration of 20 ‰ (eq. to the EC value in the nutrient solution of 38.0 dS/m), while *T. orientalis* had salinity tolerant threshold at 10‰.

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