



## Morpho-physiological Response of *Gliricidia sepium* to Seawater-induced Salt Stress

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### Abstract

Soil degradation due to the contamination of excessive saline water has been threatening soil health vis a vis plant productivity worldwide. *Gliricidia sepium*, a fertilizer tree, has come into limelight in recent decades in Bangladesh due to its potency in improving soil fertility. However, study on its suitability in coastal areas alongside identifying the salt-endurance limit is still lacking. Therefore, morpho-physiological attributes of *G. sepium* under seawater-induced different levels of salt stress were analyzed in a pot experiment from March to May 2018 to gain an insight into its salt-adaptive mechanisms. Results revealed that seawater-induced salinity negatively affected the growth-related attributes, such as plant height, fresh weight of shoots, dry weights of shoots and roots, and leaf area. The reduction of growth was coincided with reductions in photosynthetic pigments and relative water content. Interestingly, salt tolerance index was not decreased in parallel with increasing dosages of seawater, indicating the salt tolerance capacity of *G. sepium*. Furthermore, enhanced accumulation of proline increased the osmoprotective capacity of *G. sepium* in order to overcome the salt-induced osmotic stress. The results of the present study concluded that *G. sepium* might be suitable for growing in the salt prone areas ranging 20 – 40 dSm<sup>-1</sup>.

**Keywords:** Fertilizer tree, growth parameters, malondialdehyde, proline, photosynthetic pigments, soil salinity, salt tolerance index.

### 1. Introduction

Most of the parts of Bangladesh are more or less vulnerable to climatic menaces, but the coastal regions are predominantly very sensitive due to specific geo-climate and phylogenic activities. Bangladesh has a coastal area of 2.85 million ha, of which about 1.0 million ha along the coastal belt is under intimidation of different magnitudes of salinities (Rahman *et al.*, 2016). Furthermore, sea-level rise provokes the salinity intrusion in the coastal plains causing demolition of the

coastal ecosystems by degrading soil fertility, fresh water bodies and regeneration aptitude of mangroves (Rahman *et al.*, 2017). High salinity causes multifarious co-operative events that adversely affect plant growth and development, and consequently yields, resulting in reduction of agricultural outputs by billions of dollars per annum (Song and Wang, 2014; Tahjib-Ul-Arif *et al.*, 2018; Abdel Latef *et al.*, 2019). Salt stress can trigger hyper-osmotic stress by reducing soil water availability and ion toxicity by accumulating excessive Na<sup>+</sup> in the cells, which

severely impede common metabolic and physiological functions, leading to plant death in severe cases (Rajput *et al.*, 2016; Acosta-Motos *et al.*, 2017).

Apart from salinity problem, the organic matter content in Bangladesh soils is less than 1%, ranging between 0.05 and 0.9% in most cases, which also restrains the agricultural productivity of Bangladesh (Biswas and Naher, 2019). Agriculture is intimately associated with food security and acts as a mainstay of Bangladesh's economy, which employs nearly half of the total population and contributes 14.23% to the gross domestic product (BER, 2019). The challenges to sustainable land and/ or agricultural management in Bangladesh are twofold. Firstly, to boost crop production and to maintain the soil quality of the agricultural lands, secondly is to the improvement of degraded land in an environmentally sustainable way (Karim *et al.*, 2014). In this context, adoption of climate-smart organic agriculture, including crop residue recycling, legume plant inclusion in the cropping sequence or as intercrops, green leaf manuring and add-farmyard manure as supplementation or alternation of chemical fertilizers will be a propitious option to improve the soil health and crop productivity (Srinivasarao *et al.*, 2011).

*Gliricidia sepium* (*G. sepium*), is a fast-growing medium sized (10-15 m) leguminous tree belonging to the family Fabaceae. It is one of the best-known multipurpose trees originated from Central America and Mexico, but has also widely spread to West Africa, West Indies, Southern Asia and tropical America (Solangi *et al.*, 2010). It adapts very well in a wide range of soils ranging from eroded acidic (pH 4.5-6.2) soils, fertile sandy soils, heavy clay, calcareous limestone and alkaline soils (Wani *et al.*, 2009). The tree is used for timber, firewood, hedges, medicinal purpose, charcoal, live fence, plantation shade, poles, soil stabilization, and even as mouse killer (Srinivasarao *et al.*, 2011). However, *G. sepium* has come into limelight in recent decades to solve the soil fertility problem due to its capacity of improving organic matter

content in the soil (Beedy *et al.*, 2010; Makumba, 2003; Kwesiga *et al.*, 2003). Furthermore, it also improves the water infiltration into the soil, increases water-holding capacity of the soil, reduces soil erosion, restores and improves soil quality, and ultimately increases the crop yields (Diouf *et al.*, 2017). Growing *Gliricidia* plants on farm *aile* serves dual purpose as they produce green leaf manure that are rich in nitrogen (N) and helps in conserving soil through reduced soil erosion (Rao *et al.*, 2009). It is worth mentioning that application of 1 t ha<sup>-1</sup> *Gliricidia* leaf manure provides 21 kg N, 2.5 kg P, 18 kg K, 85 g Zn, 164 g Mn, 365 g Cu, 728 g Fe besides considerable quantities of S, Ca, Mg, B, Mo etc. (Srinivasarao *et al.*, 2011). Farmers should therefore be encouraged to grow *Gliricidia* on a farm *aile* of fields for use in crop production, which is a sustainable means of maintaining soil fertility along with reducing the pressure on chemical fertilizers that are environmentally perilous.

Therefore, exploration of *G. sepium* towards calamitous areas in Bangladesh will be one of the auspicious options to serve the dual role, such as restoration of degraded soils and boost agricultural yield in an organic way. Unfortunately, being a new species in Bangladesh (Hossain *et al.*, 1996), salinity tolerance limit alongside morpho-physiological response of *G. sepium* are not yet well understood. Therefore, the present study was aimed to determine the salt tolerance limits as well as appraise the effect of seawater induced salt stress on the morpho-physiological features of *G. sepium* plants.

## 2. Materials and Methods

### 2.1. Study location, plant materials and growth conditions

A pot experiment was conducted at the research field of the Department of Agroforestry and Environment, Bangabandhu Sheikh Mujibur Rahman Agricultural University, Bangladesh (24° 09' N; 90° 26' E) for a period of 3 months

(March to May 2018). The study site is 8.2 m above the sea level and characterized by a sub-tropical climate with hot summer and mild winter. The minimum and maximum temperatures of the research area were fluctuated between 17 to 23°C and 28 to 39°C, respectively, during the experimental period. Healthy *G. sepium* seeds were surface-sterilized in 5% (v/v) sodium hypochlorite solution containing 0.2% (v/v) Tween-20 for 30 min followed by five times washed with distilled water (dH<sub>2</sub>O). Subsequently, the surface-sterilized seeds were incubated in dH<sub>2</sub>O at 26°C in the dark for 24 h. The seeds were then transferred to small polythene bags containing 200 g of soils. Twenty-four days old *G. sepium* seedlings of uniform size were transplanted into each pot (23 cm in height and 28 cm in diameter). The pots were previously filled with sandy loam soil mixture that was prepared by mixing oven-dried soil and cow dung at the ratio of 4:1 (in weight basis). However, the soils used was also treated with formaldehyde (2.5 mL added in 100 mL of water) via spraying at the rate of 3.6-4.2 L of solution per m<sup>3</sup> of soils to curtail the probability of developing soil-borne diseases. Subsequently, mix thoroughly and kept the soil covered for 24 h. After removing odor of formaldehyde, the soils were used to fill the pots. In addition to cow dung, one liter of liquid fertilizer containing 1% of-urea, triple super phosphate and potassium sulphate was applied once to each pot on 20 days old-transplanted seedlings.

### 2.2. Treatments composition

After transplanting the seedlings into pots, the plants were irrigated with tap water for 6 days for adaptation before being exposed to seawater-induced salt stress. Seawater collected from southern coastal area (Cox's Bazar sea beach) of Bangladesh (electrical conductivity of 69.6 dS m<sup>-1</sup>, and Na<sup>+</sup>, K<sup>+</sup>, Ca<sup>2+</sup> and Mg<sup>2+</sup> concentrations of 578.70, 20.38, 19.90 and 66.08 mmol L<sup>-1</sup>, respectively) was diluted with tap water to formulate different levels of salt treatments (20, 40 and 60 dS m<sup>-1</sup>) and the tap water was used as control treatment. For imposing salt stress, the

uniformly grown plants were irrigated with the diluted seawater (300 mL) every day for a period of 90 days. The control plants were irrigated with tap water. Root and shoot samples were harvested at 90<sup>th</sup> days of salt treatments for determining various morpho-physiological and biochemical parameters of *G. sepium*.

### 2.3. Determination of growth parameters

Plant height and leaf area were measured with a linear scale and following the equation of Carleton and Foote (1965), respectively. For determining biomasses of shoots and roots, plants were carefully uprooted at day 90<sup>th</sup> of salt stress imposition and washed with distilled water to remove adherent soil. Fresh weights (FWs) of shoots, dry weights (DWs) of shoots and roots were measured following the method described by Mostofa *et al.*, (2015). Salt tolerance index (STI) was calculated following the formula of Rahman *et al.* (2017).

### 2.3. Determination of leaf relative water content, chlorophyll contents

The fresh, turgid and dry weights of the leaves were used to determine leaf relative water content (RWC) according to the formula described by Rahman *et al.*, (2017). Chlorophyll (Chl) and carotenoids (Car) contents of freshly collected leaf samples were determined using 80% acetone as previously described by Misratia *et al.*, (2013).

### 2.4. Contents of proline

The levels of proline (Pro) in shoots were determined following the methods described by Bates *et al.*, (1973).

### 2.5. Electrolyte leakage and lipid peroxidation

Electrolyte leakage (EL) and the content of lipid peroxidation product [malondialdehyde (MDA)] in shoots were determined according to Lutts *et al.*, (1996) and Tayebimeigooni *et al.*, (2012), respectively.

### 2.5. Statistical analysis

The data were subjected to a one-way analysis of variance (ANOVA), following randomized block

design and different letters indicate significant differences among treatments at  $P < 0.05$ , according to least significant difference test (LSD) using Statistix 10 package. Data presented are means  $\pm$  standard errors (SEs) of three biological replications ( $n=3$ ) for each treatment.

### 3. Results and Discussion

#### 3.1. Effects of salinity on plant height, FW of shoots, DW of shoots and roots, STI and leaf area of *G. sepium*

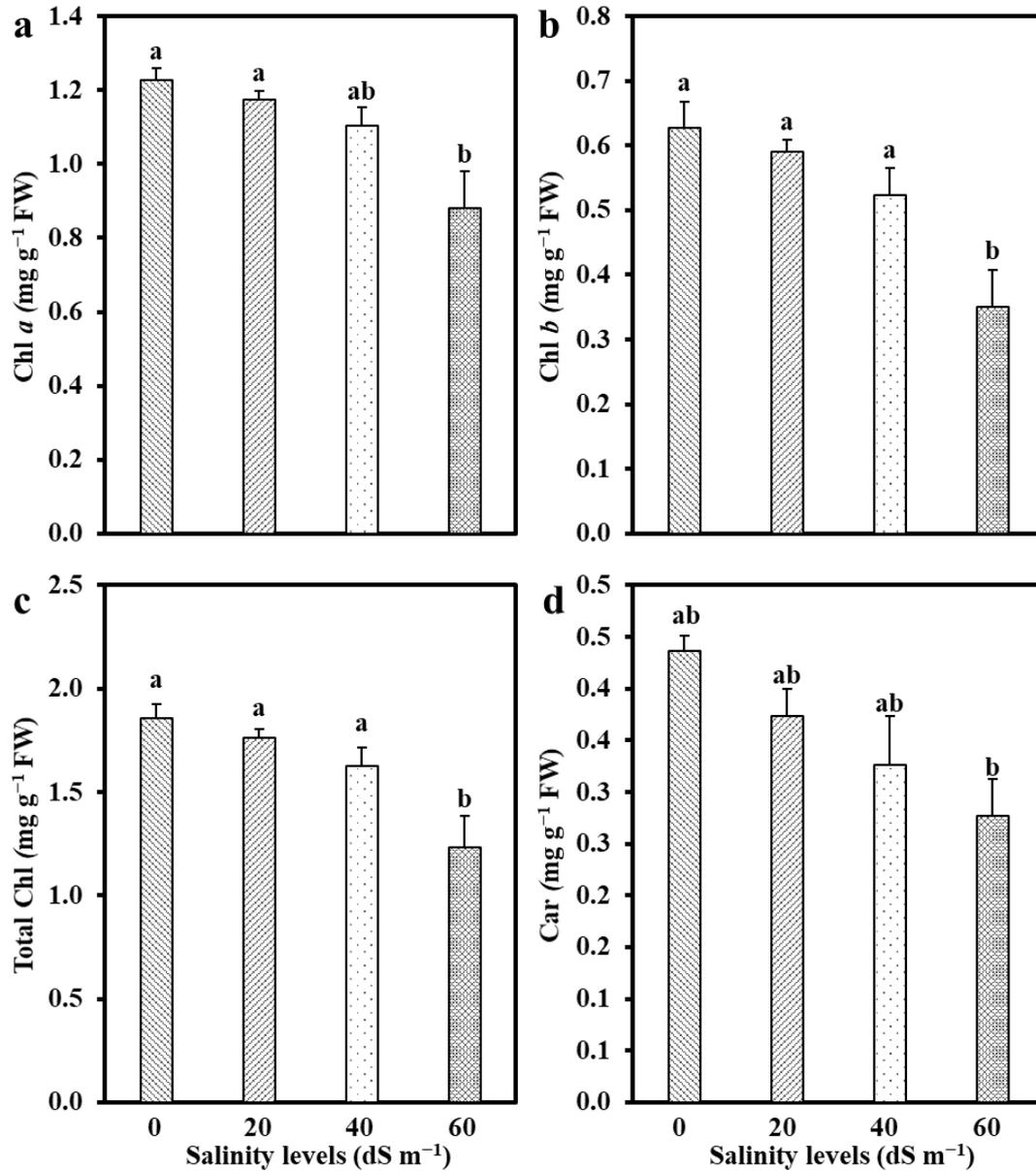
Salinity had considerable negative effects on plant height. This stress caused a significant reduction of plant height by 16.63, 25.94 and 35.26% at day 90<sup>th</sup> upon exposure of plants to 20, 40 and 60 dS m<sup>-1</sup> salinity levels, respectively, as compared to the respective control values in *G. sepium* (Table 1). Similarly, shoot FWs was significantly attenuated by 46.74, 65.48 and 77.77% upon exposure of 90<sup>th</sup> day salt treatments at the level of 20, 40 and 60 dS m<sup>-1</sup> salinity levels, respectively, as compared to the respective control values (Table 1). Nevertheless, the negative consequences of salinity on growth performance of *G. sepium* were assessed by determining DW of shoots and roots. DW of salt-stressed shoots reduced by 30.72, 45.10 and 47.71%, whereas, root DW by 41.92, 65.38 and 73.08% at 20, 40 and 60 dS m<sup>-1</sup> salinity levels, respectively, compared to that of control. STI, a reliable criterion for salt tolerance (Abbas *et al.*, 2013), was also differentially

affected by salt stress. STI decreased by 46.87% at the highest dosage of 60 dS m<sup>-1</sup> seawater and STI obtained 66.83 and 50.55% at 20 and 40 dS m<sup>-1</sup>, respectively (Table 1). Leaf area is the most sensitive growth parameter in response to salt stress (Qados, 2011). Reduction of leaf area progressively augmented with increasing salinity levels, and leaf area was found to reduce by 49.01, 51.49 and 57.55 % at day 90<sup>th</sup> in comparison to control upon application of 20, 40 and 60 dS m<sup>-1</sup> salinity levels to plants, respectively (Table 1). The attenuation of growth-related parameters under high salinity clearly manifested that salt stress interferes various physiological and metabolic processes of plant upon which growth relies on (Table 1). In general, plant growth is intricately connected to meristem activity, cell division and expansion, carbon fixation, as well as uptake of water and nutrients (Pan *et al.*, 2016; Rahman *et al.*, 2016; Rahman *et al.*, 2018). All of these processes are rigorously affected by high salinity, resulting in reduced growth performance of plants, which is further supported by the findings of Rahman *et al.*, (2018) in *Achras sapota* plant. However, the reduced leaf area observed in the salt-exposed plants (Table 1) might help in minimizing the water loss by transpiration, which in turn can favor the retention of toxic ions in the roots and limiting the translocation of these ions to the aerial parts of the plant (Theerawitaya *et al.*, 2015; Rangani *et al.*, 2016; Rahman *et al.*, 2018).

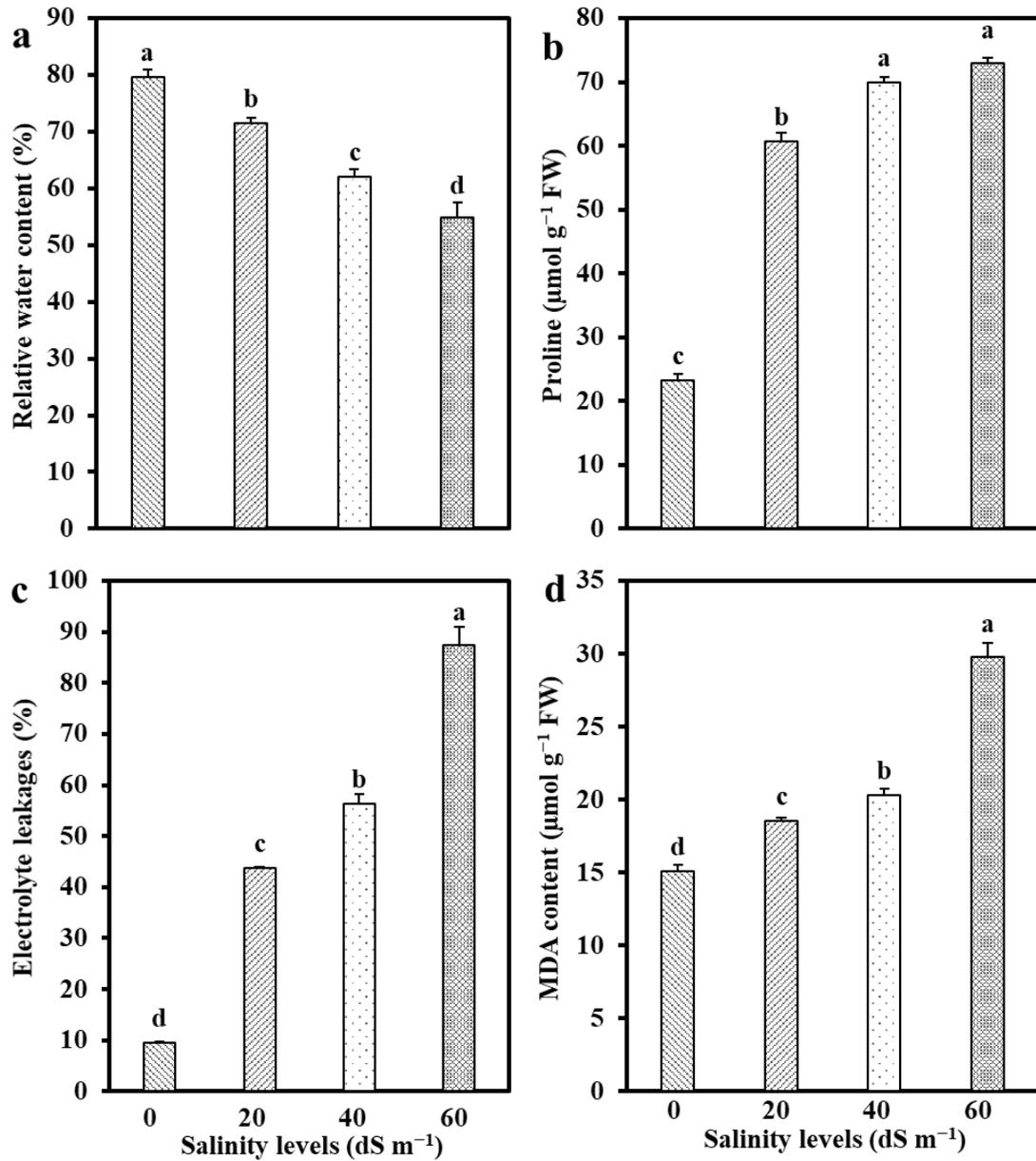
**Table 1.** Effects of salinity on plant height, shoot fresh weight (FW), shoot and root-dry weight (DW), salt tolerance index (STI) and leaf area in *G. sepium* plants after 90 days exposure

Treatments	Plant height (cm)	Shoot FW (g)	Shoot DW (g)	Root DW (g)	STI	Leaf area per plant (cm <sup>2</sup> )
Control	150.33 $\pm$ 8.83 <sup>a</sup>	103.13 $\pm$ 1.45 <sup>a</sup>	12.75 $\pm$ 0.14 <sup>a</sup>	3.47 $\pm$ 0.35 <sup>a</sup>	-	1618.72 $\pm$ 20.49 <sup>a</sup>
20dS/m	125.33 $\pm$ 3.76 <sup>b</sup>	54.93 $\pm$ 1.98 <sup>b</sup>	8.83 $\pm$ 0.30 <sup>b</sup>	2.01 $\pm$ 0.01 <sup>b</sup>	66.87 $\pm$ 1.88 <sup>a</sup>	825.40 $\pm$ 21.86 <sup>b</sup>
40dS/m	111.33 $\pm$ 2.03 <sup>bc</sup>	35.6 $\pm$ 1.41 <sup>c</sup>	7.00 $\pm$ 0.14 <sup>c</sup>	1.20 $\pm$ 0.23 <sup>bc</sup>	50.57 $\pm$ 0.53 <sup>b</sup>	785.20 $\pm$ 30.24 <sup>b</sup>
60dS/m	97.33 $\pm$ 4.06 <sup>c</sup>	22.93 $\pm$ 1.97 <sup>d</sup>	6.67 $\pm$ 0.44 <sup>c</sup>	0.93 $\pm$ 0.13 <sup>c</sup>	46.87 $\pm$ 2.68 <sup>b</sup>	687.20 $\pm$ 11.02 <sup>c</sup>

Values are means  $\pm$  standard errors of three biological replications ( $n = 3$ ). Different superscripted letters within a column indicate statistically significant differences among the treatments according to a least significant difference test ( $P < 0.05$ ). FW, fresh weight; DW, dry weight; STI, salt tolerance index.



**Figure 1.** Effects of salinity (0, 20, 40 and 60 dS m<sup>-1</sup>) on (a) Chl a, (b) Chl b, (c) Chl (a+b) (d) Car content of *Gliricidia sepium* after 90 days exposure. Bars represent standard errors of the means ( $n = 3$ ). Different letters indicate significant differences among the treatments at  $P < 0.05$ , according to a least significant difference test. Chl, Chlorophyll; Car, Carotenoid; FW, fresh weight.



**Figure 2.** Effects of salinity on (a) relative water content, (b) proline, (c) electrolyte leakage (d) malondialdehyde (MDA) content of *Gliricidia sepium* after 90 days exposure (0, 20, 40 and 60 dS m<sup>-1</sup>). Bars represent standard errors of the means ( $n = 3$ ). Different letters indicate significant differences among the treatments at  $P < 0.05$ , according to a least significant difference test. FW, fresh weight.

### 3.2. Effects of salinity on photosynthetic pigment

Irrigation of *G. sepium* plants with diluted seawater caused gradual and significant decreases in the contents of Chl *a* (by 4.35, 10.06 and 28.26% at day 90<sup>th</sup>), Chl *b* (by 5.86, 16.50 and 44.15% at day 90<sup>th</sup>), total Chls (by 5.21, 12.39 and 33.75% at day 90<sup>th</sup>) and carotenoid content (by 14.52, 25.19 and 36.64% at day 90<sup>th</sup>) upon imposition of 20, 40 and 60 dS m<sup>-1</sup> salinity, respectively, compared with plants irrigated with tap water (Fig. 1). Photosynthetic efficiency of a plant determines the overall performance, which is emulated by growth and biomass parameters. In the current study, the levels of Chl *a* and *b*, as well as total Chls, were progressively declined with the rise of salinity level and exposure time, indicating that the photosynthetic performance of *G. sepium* was hampered, which contributed to the decrease in plant biomass (Fig. 1; Table 1).

The reduction in Chl levels and consequent biomass loss were evident in other studies under salt stress, and the possible cause was attributed to the direct effects of accumulated Na<sup>+</sup> and ROS (Qin et al., 2010; Mostofa et al., 2015). Chl *a* is the most abundant and integral part of the light-harvesting complex, whereas Chl *b* as an accessory pigment acts indirectly in photosynthesis by transmitting photons to the Chl *a* (Chen, 2014; Gururani et al., 2015). Thus, a higher level of Chl *a* than Chl *b* at all concentrations of seawater (Fig. 1a, b) might have contributed to the improved efficiency of *G. sepium* in resisting salt-induced loss of photosynthetic capacity (Table 1), as was reported in *Atriplex helimus* by Bendaly et al., (2016) and in *Acacia auriculiformis* plant by Rahman et al., (2017). Besides functioning as accessory pigments, carotenoids are known to be involved in protecting photosynthetic reaction center from chlorophyll-mediated generation of singlet oxygen (<sup>1</sup>O<sub>2</sub>) by quenching triplet states of Chls, and thus stabilizing and protecting the lipid phase of thylakoid membrane (Gururani et al., 2015).

### 3.3. Effects of salinity on leaf RWC, content of Pro, leaf EL, and MDA of *G. sepium*

In response to seawater-induced salinity, leaf RWC decreased by 10.10, 21.98 and 31.16% at day 90<sup>th</sup> under 20, 40 and 60 dS m<sup>-1</sup> salinity levels, respectively, compared to control (Fig. 2a). To understand the mechanisms associated with osmotic adjustment under salt stress, we have determined the level of Pro in *G. sepium* plants under normal and salt stress conditions. Pro content remarkably increased by 161.99, 201.95 and 214.91 % at day 90<sup>th</sup> upon imposition of 20, 40 and 60 dS m<sup>-1</sup> diluted seawater, respectively, compared to control (Fig. 2b). To assess the cell membrane integrity under salt stress, we determined the level of EL in the shoots of *G. sepium* (Fig. 2c). At day 90<sup>th</sup> of salt imposition, EL content significantly increased by 361.43 and 495.96% at 20 and 40 dS m<sup>-1</sup> salinity levels, respectively, relative to control. Nevertheless, the increment of EL levels showed the maximum 823.73% at the highest salinity dose (60 dS m<sup>-1</sup>) than that of untreated respective control. To assess the cell membrane integrity under salt stress, we determined the levels of lipid peroxidation product MDA in the shoots of *G. sepium* MDA content significantly increased by 22.83, 34.82 and 97.50% at day 90<sup>th</sup> upon imposition of 20, 40 and 60 dS m<sup>-1</sup> salinity, respectively, compared to control plants (Fig. 2d). In the present study, reduction of leaf RWC in salt-stressed plants upon exposure to salinity indicating the salt-mediated induction of physiological water deficit in *G. sepium* (Fig. 2a). However, enhanced accumulation of Pro in salt-stressed plants might help adjust to water deficit conditions (Fig. 2b). Pro is known to provide improved protection against salinity by scavenging free radicals, stabilizing membranes, proteins and enzymes, and maintaining ion homeostasis (Kishor and Sreenivasulu, 2014). Nonetheless, higher accumulation of toxic ions under salt stress lead to the generation of reactive oxygen species (ROS). ROS predominantly attacks the membrane, causing lipid peroxidation that leads to the generation of MDA and loss of essential electrolytes (Penella et al., 2016). The levels of MDA content and EL in *G. sepium*

plants enhanced to a lower extent at low and moderate salinity levels (20-40 dS m<sup>-1</sup>), where it was sharply increased at high dosage of salinity (60 dS m<sup>-1</sup>), which indicate that *G. sepium* plants could resist themselves from salt-induced membrane damage up to a certain level of salinity.

#### 4. Conclusions

Our findings concluded that exposure of *G. sepium* plants to seawater-induced salinity adversely affects the performance of the whole plant, particularly by inhibiting growth and development. Nevertheless, the adapted stress tolerance mechanisms of *G. sepium* at a salinity range of 20 – 40 dsm<sup>-1</sup> could be attributed to (1) maintained higher dry biomass, (2) maintained optimum water status in terms of elevated RWC, (3) abundance of primary photosynthetic pigment Chl *a* over accessory pigment Chl *b* to sustain favourable photosynthetic rate, and (4) accumulation of osmolyte like Pro to maintain osmoprotectant capacity under salt-stress. Nevertheless, we postulated that established plants of this species might be tolerant to salt stress higher than the level used in this study because seedlings are more sensitive to high soluble salt levels than established plants.

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