

## Altered water relations, selective nutrient uptake, and reduced Na<sup>+</sup> flux make *Halopeplis perfoliata* an obligate halophyte

Sarwat Ghulam RASOOL, Muhameed Zaheer AHMED, Abdul HAMEED, Bilquees GUI\*  
Dr. Muhammad Ajmal Khan Institute of Sustainable Halophyte Utilization, University of Karachi, Pakistan

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**Abstract:** *Halopeplis perfoliata*, a succulent halophyte of coastal marshy habitats, was grown in a greenhouse to study its adaptive responses in terms of growth, osmoregulation, and N-metabolism following one-month exposure to salinity (0, 150, 300, and 600 mmol L<sup>-1</sup> NaCl). Biomass was optimal in 150 mmol L<sup>-1</sup> NaCl with unaltered malondialdehyde (MDA) levels. On the other hand, biomass remained comparable to non-saline control with some increase in MDA in 300 and 600 mmol L<sup>-1</sup> NaCl. Osmotic potential ( $\psi_s$ ) was maintained up to 300 mmol L<sup>-1</sup> NaCl that correlated with an increase in succulence (SC) and lower water deficit (WD), whereas sap  $\psi_s$  was lowest in 600 mmol L<sup>-1</sup> NaCl along with lower SC and high WD. Sodium level gradually increased in shoot and was higher than the root. Potassium deficiency was not observed up to high salinity. Both soluble sugars and nitrogen isotope ( $\delta^{15}\text{N}$ ) increased transiently in 300 mmol L<sup>-1</sup> NaCl. Hence, modulations in water relation, selective nutrient uptake, and controlled Na<sup>+</sup> flux appear important for salinity tolerance of *H. perfoliata*.

**Key words:** Ion toxicity, nitrogen metabolism, nutrient homeostasis, osmotic adjustment, salt marsh, selective membrane transport

### 1. Introduction

Coastal salt marshes are a highly productive ecosystem of the world (Tran et al., 2020) and harbor unique halophyte vegetation with specialized adaptations that produces enormous biomass despite high seawater salinity (Rozema and Schat, 2013; Lima et al., 2020). These salt marshes possess numerous ecological and economic importance, for instance, they provide habitat for coastal fauna and flora (Tran et al., 2020). However, these habitats are subject to various anthropogenic activities (Yasseen and Al-Thani, 2007; Ramadan et al., 2013). For example, frequent construction activities in coastal regions pose a serious threat to these marshes (Tran et al., 2020). Extreme variations in various abiotic conditions such as soil salinity cause additional stress for plants (Gul and Khan, 2006; Camacho-Sanchez et al., 2020). Initially, soil salinity inhibits plant growth via osmotic stress, which is then followed by ion toxicity (Munns and Tester, 2008). Osmotic stress occurs by low soil osmotic potentials that restricts water acquisition/retention and ionic toxicity results from high cellular levels of potentially toxic Na<sup>+</sup> and Cl<sup>-</sup> ions (Munns and Tester, 2008; Elnaggar et al., 2020).

Halophytes of marshy habitat can endure as high as 1000 mmol L<sup>-1</sup> NaCl during their growth (Gul and Khan, 2006; Hameed et al., 2012). In addition, growth of coastal

marsh halophytes appears to be promoted in ranges from 200 to 400 mmol L<sup>-1</sup> NaCl in contrast to xero-halophytes that showed optimal performance below 200 mmol L<sup>-1</sup> NaCl (Yue et al., 2012). In general, marshy vegetation has high succulent nature and regulate tissue osmotic potential with the help of ion compartmentalization in cell vacuoles (Munns and Tester, 2008; Osman et al., 2020). High Na<sup>+</sup> contribution in tissue osmotic potential is a prominent feature of marshy vegetation. For example, many succulent dicots of the coastal marshes such as *Arthrocnemum macrostachyum* (Redondo-Gómez et al., 2010), *Salicornia fruticosa* (Marco et al., 2019) and *Sarcocornia quinqueflora* (Ahmed et al., 2021) show high leaf/shoot succulence and tissue Na<sup>+</sup> content. Moreover, the accumulation of soluble carbohydrates and nitrogenous compounds (quaternary ammonium compounds, amino acids, polyols, and proteins) also serve as osmolytes (Pardo-Domènech et al., 2016; Flowers and Colmer, 2015). However, high salinity decreases the nitrogen content in tissues as well as the  $\delta^{15}\text{N}$ , and frequently a positive relationship between  $\delta^{15}\text{N}$  and biomass/yield has been reported (Yousfi et al., 2012). Most succulent halophytes have high shoot/root Na<sup>+</sup> in saline conditions, while sequester toxic ions (Na<sup>+</sup> and Cl<sup>-</sup>) in the vacuoles to reduce ion toxicity and intact cell metabolisms (Flowers and Colmer, 2015). In general,

\* Correspondence: bilqueesgul@uok.edu.pk

succulent halophytes have a poor capacity to retain tissue nutrient homeostasis while increasing  $\text{Na}^+/\text{K}^+$  ratios under high salinity (Munns and Tester, 2008).

*Halopeplis perfoliata* Forssk. is a  $\text{C}_3$  perennial halophyte, belonging to the family Amaranthaceae and is widely distributed in the Mediterranean region, North Africa and in Southwest Asia to Central Asia, specifically at sea-shores from the Red Sea, Sinai Peninsula, Arabian Peninsula, to southwestern Pakistan (Balochistan) (Hedge, 1997). *Halopeplis perfoliata* is usually found close to the sea-shores of the Red Sea area, in the region of Jizan. This species flourishes in silty and slippery soil having high water content and co-occurs with many succulent halophytes such as *Arthrocnemum macrostachyum*, *Halocnemum strobilaceum*, *Salicornia europaea* (Alfarhan et al., 2005). *Halopeplis perfoliata* has numerous ecological and economic utilities. This plant serves as the primary producer of intertidal zones, provides habitat for coastal fauna (Pilcher et al., 2003), and has a high potential for sand dune stabilization (Zreik, 1990). Öztürk et al. (2019) highlighted the potential of *H. perfoliata* as animal fodder in the coastal areas. High amounts of many medicinal compounds have also been reported in this plant (Al-Saleh et al., 1997). Information about seed germination ecology, ethno-botany, and community/population biology of this halophyte is known. Al-Zahrani and Hajar (1998) reported that this plant can tolerate high salinity up to  $510 \text{ mmol L}^{-1} \text{ NaCl}$ . Recently, Rasool et al. (2019) reported that maintenance of photosynthesis, and the antioxidant defense system play key roles in salinity tolerance of *H. perfoliata*. However, detailed information about aspects like water relations, ion flux, osmolyte accumulation, nutrient homeostasis is yet to be studied to better understand the high salinity tolerance of this important halophyte. Therefore, the aim of this study was to investigate the effects of different salinity levels on vegetative growth, water relations ion relations, and  $\delta^{15}\text{N}$  levels of *H. perfoliata* seedlings.

## 2. Materials and methods

### 2.1. Seed collection

Inflorescence of *H. perfoliata* were collected from a coastal marsh of Jizan, Saudia Arabia (longitude,  $42.5679^\circ$ ; latitude,  $16.8894^\circ \text{ N}$ ; altitude, 40 meter) in December 2012. Seeds were collected randomly from a large number ( $> 75$ ) of plants. Seeds were cleaned and surface-sterilized by using 0.82% sodium hypochlorite solution for 1 min (Hameed et al., 2013) and were stored at room temperature ( $25 \pm 5^\circ \text{ C}$ ) in sealed transparent plastic bags until further use.

### 2.2. Growth conditions

Seedlings were raised in sandy soil-containing plastic pots (25 cm height, 19 cm diameter). Plants (about 150) were grown in semi ambient conditions (temperature: ranging

from  $32^\circ \text{ C}$  to  $40^\circ \text{ C}$ ; relative humidity ranging from 40% to 60% and  $\text{PAR} \sim 600 \mu\text{mol m}^{-2} \text{ s}^{-1}$ ) in greenhouse. Plants were sub-irrigated (size of irrigation trays were 12 cm depth, 30 cm diameter) with 2 L of half strength Hoagland's nutrient solution (Epstein, 1972) (nitrogen, 112 ppm; phosphorus, 31 ppm; potassium, 117 ppm) prepared in tap water (containing 598 ppm sodium). Seedlings with the height of  $\sim 10$  cm were selected for experimental treatments [16 plants per treatment ( $0 \text{ mmol L}^{-1} \text{ NaCl}$  – nonsaline,  $150 \text{ mmol L}^{-1} \text{ NaCl}$  – low,  $300 \text{ mmol L}^{-1} \text{ NaCl}$  – moderate and  $600 \text{ mmol L}^{-1} \text{ NaCl}$  – high); 4 plants for each set of analysis (growth/  $\delta^{15}\text{N}$ , water relation, ion/soluble sugars, and MDA)] at the rate of  $50 \text{ mmol L}^{-1} \text{ NaCl}$  after every 12 h in such a way that all salinity concentrations were achieved on the same day. The volume of experiment solution (2 L per tray) was maintained in the sub-irrigation trays by adding water, while salinity levels were monitored daily with the help of a hand-held salinity refractometer (model and company name). Plants were harvested after 5 weeks of final salinity levels achieved.

### 2.3. Growth parameters and damage marker

Whole plant shoot and root fresh mass ( $F_M$ ) was determined ( $n = 4$ ) of each treatment by using analytical weighing balance (Mettler Toledo-PL601-S) immediately after harvest. Same shoot and root samples were oven-dried at  $60^\circ \text{ C}$  for 48 h and then weighed for the estimation of dry mass ( $D_M$ ).

Malondialdehyde (MDA, a common oxidative damage marker) content was quantified in Trichloroacetic acid (TCA) extracts of shoot samples ( $n = 4$ ) collected from the second node of each treatment. TCA extracts were prepared by homogenizing finely ground shoot samples (0.5 g) in ice-cold 1% TCA (5 ml), followed by centrifugation at  $12000 \times g$  for 15 min at  $4^\circ \text{ C}$ . The supernatant was stored at  $-80^\circ \text{ C}$  in the form of aliquots prior to biochemical assays, which were conducted at  $25^\circ \text{ C}$ . TCA extract (0.5 mL) was mixed with of TCA [20% (w/v); 0.5 mL] containing 2-thiobarbituric acid [0.5% (w/v)] than heated at  $95^\circ \text{ C}$  for 30 min in a shaking water bath. The reaction was terminated in an ice bath, followed by centrifugation at  $12000 \times g$  for 10 min at  $4^\circ \text{ C}$ . The absorbance of the supernatant was measured at 532 nm and 600 nm (Heath and Packer, 1968) by using a spectrophotometer (Beckman DU-530, Beckman Coulter Inc., USA).

### 2.4. Water relations

#### Shoot osmotic potential

Shoots samples ( $n = 4$ ) were collected from the second node of plants from each treatment immediately after the final harvest. Shoot osmotic potential ( $\psi_s$ ) was estimated in tissue sap which was obtained using MARKHART leaf press (LP 27; Wescor Inc., Logan, UT, USA). A vapor pressure osmo-meter (VAPRO-5520; Wescor Inc., Logan, UT, USA) was then used to measure sap osmolality.

Briefly, 10  $\mu\text{L}$  sap was applied on a small cleaned paper disc placed in the apparatus chamber. Sample chamber was then sealed and osmolality (as  $\text{mOsmol kg}^{-1}$ ) was noted, which was then converted into osmotic potential ( $\psi_s$ ) by applying Van't Hoff equation according to Kramer and Boyer (1995).

#### Shoot succulence

Shoot succulence (SC) was calculated for each plant ( $n = 4$ ) from different treatment by using following formulae:

$$\bar{SC} = \frac{(F_M - D_M)}{(D_M)}$$

Where  $F_M$  and  $D_M$  represent fresh and dry matter.

#### Shoot water deficit

Shoot water deficit (WD) was determined by using following formulae:

$$WD = \frac{(AF_{MC} - AD_{MC})}{AF_{MC}} - \frac{(F_{MT} - D_{MT})}{F_{MT}} \times 100$$

Where  $AF_{MC}$  and  $AD_{MC}$  represent average  $F_M$  and  $D_M$  of non-saline control while  $F_{MT}$  and  $D_{MT}$  for each plant ( $n = 4$ ) of salinity treatment, respectively.

## 2.5. Ion relations

#### Tissue ion flux

Whole plant shoots and root samples ( $n = 4$ ) of different treatments were used to determine the cation concentration. Press sap of fresh plant material was obtained immediately after harvest using MARKHART leaf press (LP 27; Wescor Inc., Logan, UT, USA) to determine the cations ( $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Ca}^{++}$  and  $\text{Mg}^{++}$ ) concentrations in shoot and root tissues by atomic absorption spectrometry (AA-700; Perkin Elmer, CA, USA).

#### Selective absorption and selective transport of nutrient

The selective absorption (SA) (from medium to root) and selective transport (ST) ratio (from root to shoot) of different nutrients ( $\text{K}^+$ ,  $\text{Ca}^{++}$  and  $\text{Mg}^{++}$ ) over  $\text{Na}^+$  were calculated by using the formula of Ning et al. (2015).

$$SA [x / \text{Na}^+] = \frac{[x / (x + \text{Na}^+)]_{\text{root}}}{[x / (x + \text{Na}^+)]_{\text{medium}}}$$

$$ST [x / \text{Na}^+] = \frac{[x / (x + \text{Na}^+)]_{\text{shoot}}}{[x / (x + \text{Na}^+)]_{\text{root}}}$$

Where  $x$  indicates  $\text{K}^+$ ,  $\text{Ca}^{++}$  and  $\text{Mg}^{++}$  ions.

## 2.6. Soluble sugars

Soluble sugar (SS) content in shoot tissues ( $n = 4$ ; same samples that used for ion flux measurements) from different treatments was estimated by Anthrone's reagent method of Yemm and Willis (1954). Tissue sap (500  $\mu\text{L}$ ; same extracted sap that used for ion flux measurements) was mixed with anthrone reagent and incubated for

exactly 11 min in a boiling water bath. The reaction was terminated in an ice bath. Absorbance was read at 630 nm (Yemm and Willis, 1954). Glucose was used to prepare a standard curve.

## 2.7. Nitrogen isotope

Shoot nitrogen isotope ( $\delta^{15}\text{N}$ ) values were determined relative to atmospheric  $\text{N}_2$  (Ehleringer and Osmond, 1989). Dry shoot samples ( $n = 4$ ; same samples used to determine the  $F_M$  and then  $D_M$ ) of each treatment were ground to a fine powder by a mixer mill (Retsch GmbH, Haan, Germany). Approximately 100 mg of each sample per treatment was used to determine the  $\delta^{15}\text{N}$  by combusting the plant samples and using gas chromatography. After that, isotope values were determined by the Isoprime isotope ratio mass spectrometer (Yohannes et al., 2008).

## 2.8. Statistical analyses

One-way analyses of variance (ANOVA; SPSS, 2011) were used to determine whether salinity affected the growth and physiological parameters of *H. perfoliata* significantly. A Bonferroni post-hoc test was used to determine if the significant ( $p < 0.05$ ) differences occurred between individual treatments.

## 3. Results

### 3.1. Plant growth and tissue damage

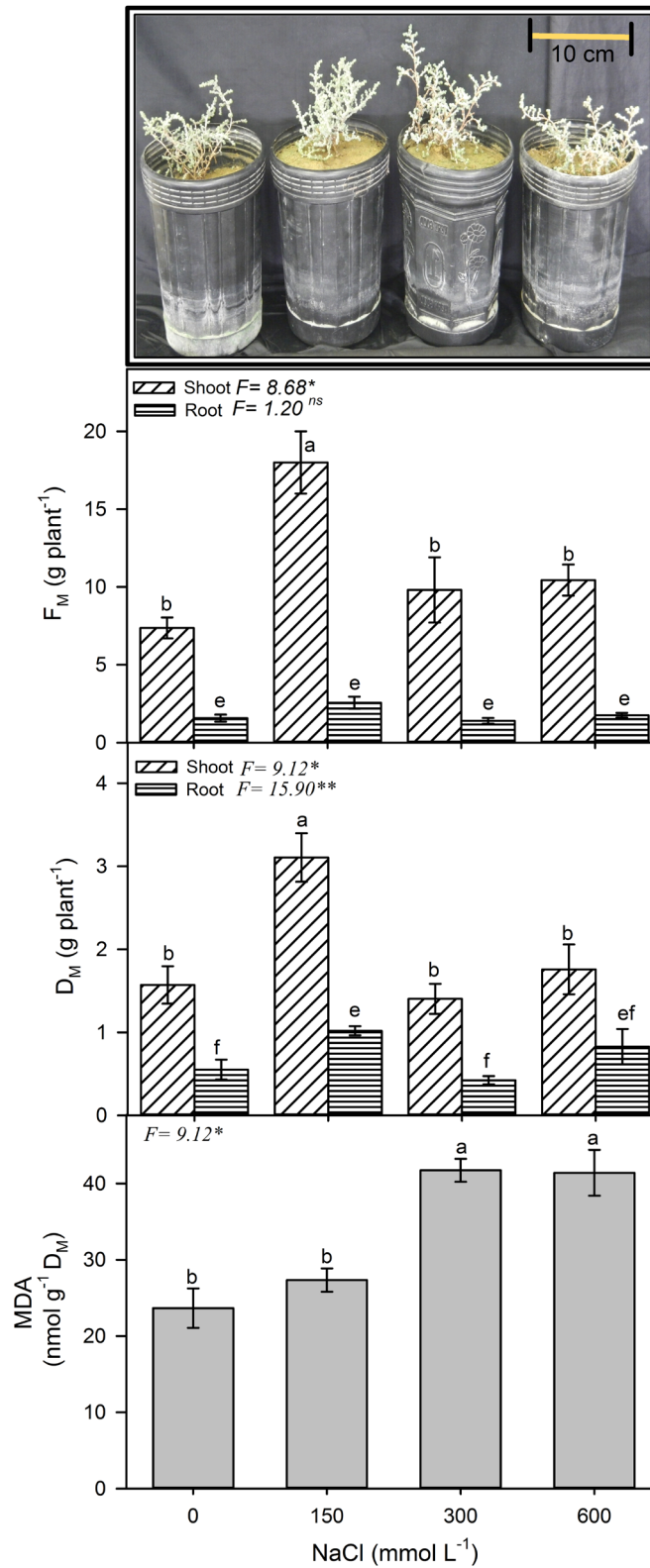
Plant growth (based on  $F_M$  and  $D_M$ ) was highest at 150  $\text{mmol L}^{-1}$   $\text{NaCl}$  compared to 0, 300 and 600  $\text{mmol L}^{-1}$   $\text{NaCl}$  treatments (Figure 1). Salinity had a significant effect ( $P < 0.05$ ) on the shoot MDA content of *H. perfoliata* (Figure 1). MDA content was unchanged at 150  $\text{mmol L}^{-1}$   $\text{NaCl}$  while increased at 300 and 600  $\text{mmol L}^{-1}$   $\text{NaCl}$ , as compared to non-saline control (Figure 1).

### 3.2. Water relations

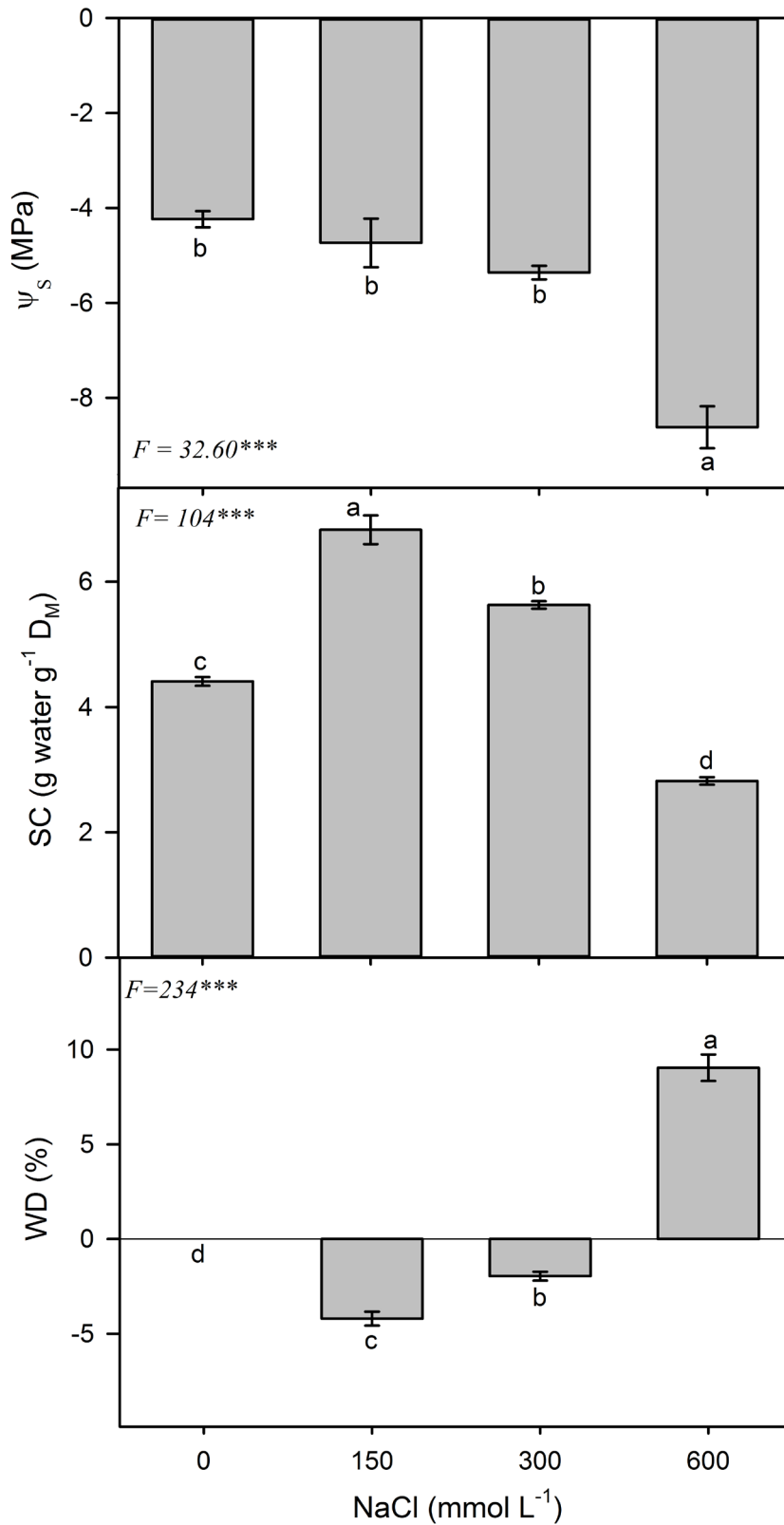
Shoot osmotic potential ( $\psi_s$ ) was increased up to 2-folds under 600  $\text{mmol L}^{-1}$  compared to control treatment, while no change was found in other treatments (Figure 2). In addition, shoot succulence (SC) was highest at 150  $\text{mmol L}^{-1}$  (Figure 2) and gradually decreased with increasing salinity (300 and 600  $\text{mmol L}^{-1}$ ; Figure 2). Shoot water deficit (WD) was only observed at 600  $\text{mmol L}^{-1}$   $\text{NaCl}$  compared to other treatments (Figure 2).

### 3.3. Ion regulation

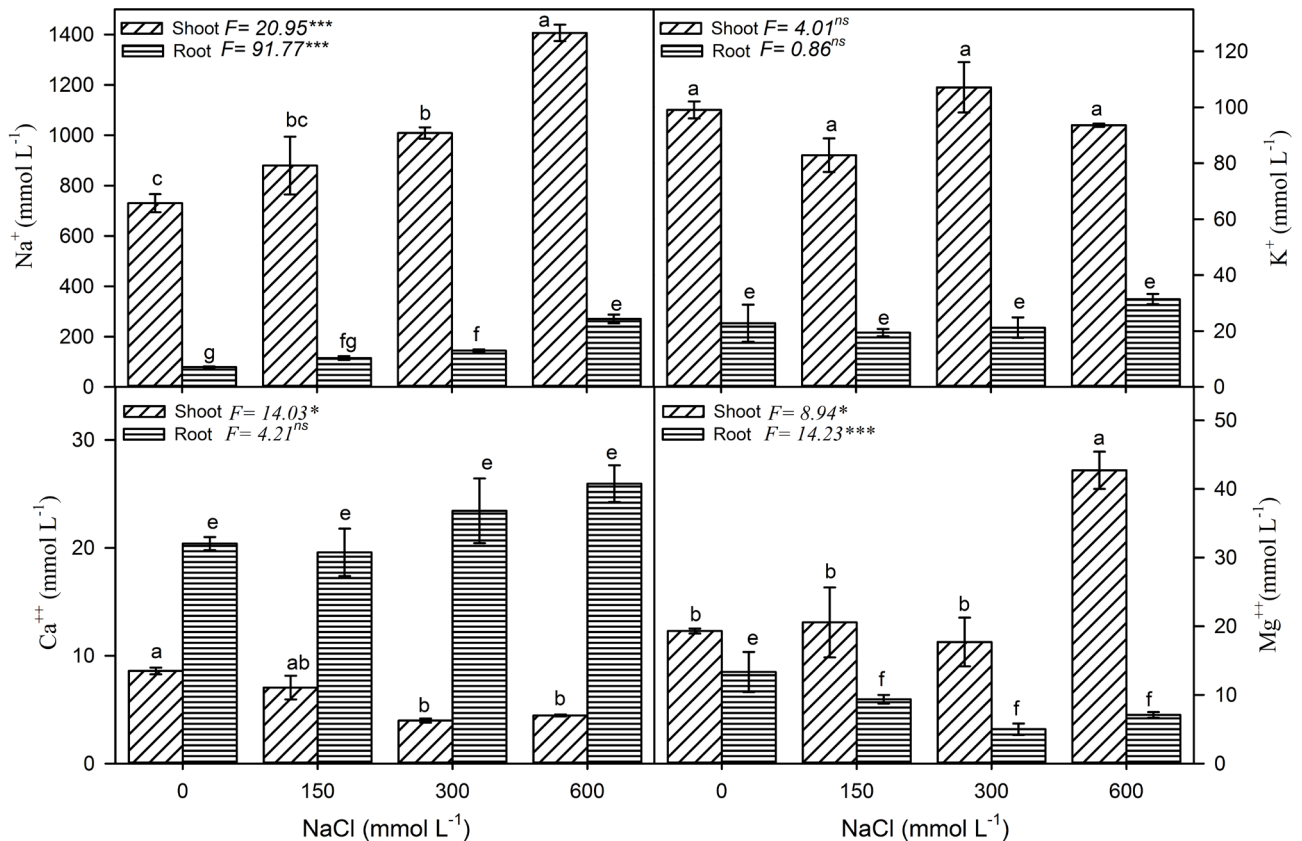
Salinity had significant effects on shoot and root  $\text{Na}^+$  and  $\text{Mg}^{++}$  ( $p < 0.05$ ) and shoot  $\text{Ca}^{++}$  ( $p < 0.05$ ) contents (Figure 3).  $\text{Na}^+$  increased linearly with increases in salinity in both tissues (shoot and root) of the plant, whereas shoot  $\text{Na}^+$  was substantially higher than root  $\text{Na}^+$  (Figure 3).  $\text{K}^+$  concentration in both shoots and roots remained unchanged by salinity increments; however, shoot  $\text{K}^+$  content was significantly higher than in roots (Figure 3).  $\text{Ca}^{++}$  decreased in shoot tissue with the increment of salinity but remained unaffected in the root (Figure 3). Shoot  $\text{Mg}^{++}$



**Figure 1.** *Halopeplis perfoliata* grown under different NaCl treatments. Shoot and root fresh mass ( $F_M$ ), dry mass ( $D_M$ ), and shoot malondialdehyde content (MDA) across different NaCl (0, 150, 300, 600 mmol L $^{-1}$ ) treatments. Bars are mean  $\pm$  standard error. Bars with different letters are significantly different from each other (Bonferroni test,  $p < 0.05$ , for the panels in which results of shoot and root are listed, letters a-c and e-g were used, respectively).



**Figure 2.** Shoot osmotic potential ( $\psi_s$ ), succulence (SC) and water deficit (WD) across different NaCl (0, 150, 300, 600 mmol L<sup>-1</sup>) treatments. Bars are mean  $\pm$  standard error. Bars with different letters are significantly different from each other (Bonferroni test,  $P < 0.05$ ).



**Figure 3.** Concentrations of cations ( $\text{Na}^+$ ;  $\text{K}^+$ ;  $\text{Ca}^{++}$ ;  $\text{Mg}^{++}$ ) in shoot and root sap of *H. perfoliata* grown under different NaCl (0, 150, 300, 600  $\text{mmol L}^{-1}$ ) treatments. Bars are mean  $\pm$  standard error. Bars with different letters are significantly different from each other (Bonferroni test,  $p < 0.05$ , for the panels in which results of shoot and root are listed, letters a-c and e-g were used, respectively).

was increased at 600  $\text{mmol L}^{-1}$  NaCl compared to 0, 150 and 300  $\text{mmol L}^{-1}$  NaCl treatments, but, in general, root  $\text{Mg}^{++}$  was lower in saline conditions compared to non-saline condition (Figure 3).

### 3.4. Nutrients availability

Shoot  $\text{Na}^+/\text{K}^+$  and  $\text{Na}^+/\text{Mg}^{++}$  increased at 600  $\text{mmol L}^{-1}$  and 300  $\text{mmol L}^{-1}$  NaCl, respectively (Figure 4). However,  $\text{Na}^+/\text{Ca}^{++}$  ratio increased gradually with increasing salinity (Figure 4).

### 3.5. Selective absorption and transport of nutrients

Selective absorption (SA) of nutrients ( $\text{K}^+$ ,  $\text{Ca}^{++}$  and  $\text{Mg}^{++}$ ) over  $\text{Na}^+$  was increased in saline treatments than non-saline control (Table). In addition, high values of SA were found at 600  $\text{mmol L}^{-1}$  NaCl (Table). Selective transport (ST) of nutrients ( $\text{K}^+$  and  $\text{Mg}^{++}$ ) over  $\text{Na}^+$  increased at 300 and 600  $\text{mmol L}^{-1}$  NaCl than non-saline control (Table). However, selective transport of  $\text{Ca}^{++}$  over  $\text{Na}^+$  unaffected from salinity (Table). In *H. perfoliata*, values of SA were higher than ST, irrespective of salinity treatment (Table).

### 3.6. Soluble sugars

Salinity had significant effect ( $p < 0.05$ ) on shoot soluble sugars (SS) of *H. perfoliata*. The SS levels significantly

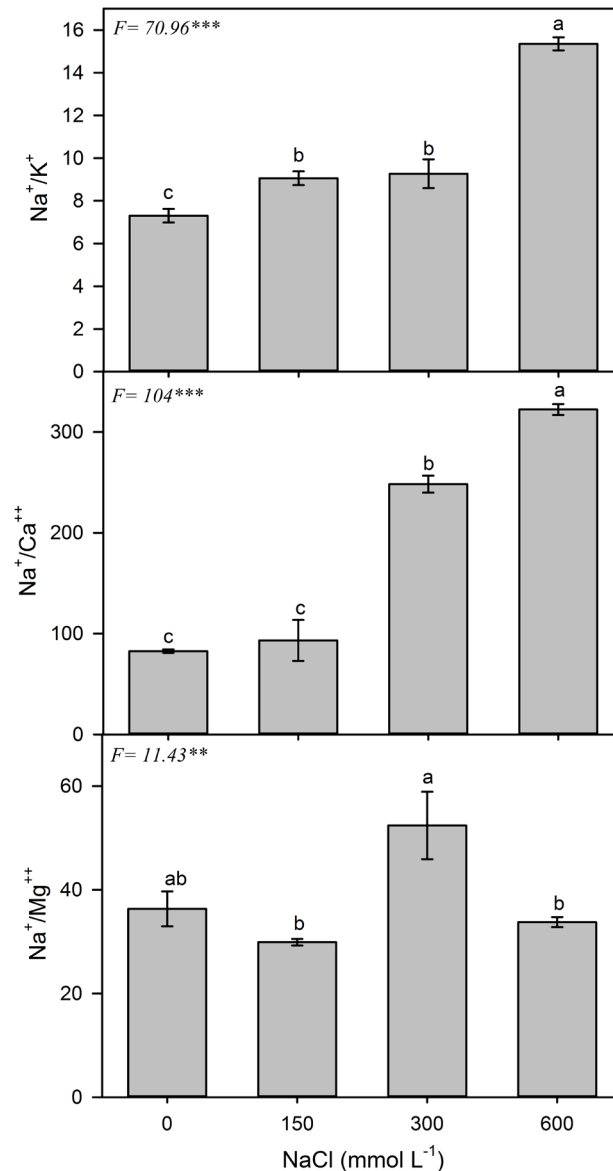
increased only in 300  $\text{mmol L}^{-1}$  NaCl treatments (Figure 5).

### 3.7. Nitrogen isotope level

Salinity had a significant effect ( $P < 0.05$ ) on the nitrogen isotope ( $\delta^{15}\text{N}$ ) of *H. perfoliata*. Values of  $\delta^{15}\text{N}$  increased at 150  $\text{mmol L}^{-1}$  and 300  $\text{mmol L}^{-1}$  NaCl treatments, while remained constant at 600  $\text{mmol L}^{-1}$  NaCl in comparison with non-saline control (Figure 5).

## 4. Discussion

Succulent halophytes in the family Amaranthaceae, subfamily Salicornioideae grow optimally in salinity (Flower and Colmer, 2008; Rozema and Schat, 2013). For example, growths of *Salicornia fruticosa* (200  $\text{mmol L}^{-1}$  NaCl; Marco et al., 2019); *S. dolichostachya* (300  $\text{mmol L}^{-1}$  NaCl; Katschnig et al., 2013) and *S. utahensis* (600  $\text{mmol L}^{-1}$  NaCl, Gul et al., 2009), *Sarcocornia quinqueflora* (200-400  $\text{mmol L}^{-1}$  NaCl; Ahmed et al., 2021) were reported to be promoted in salinity as compared to non-saline control. In the present study, the growth of *H. perfoliata* was optimal at 150  $\text{mmol L}^{-1}$  NaCl, which is associated with significantly increased shoot succulence. Increased succulence in plants



**Figure 4.** Na<sup>+</sup>/K<sup>+</sup>; Na<sup>+</sup>/Ca<sup>++</sup> and Na<sup>+</sup>/Mg<sup>++</sup> ratios in shoots of *H. perfoliata* in response to various NaCl (0, 150, 300, and 600 mmol L<sup>-1</sup>) treatments. Bars are mean ± standard error. Bars with different letters are significantly different from each other (Bonferroni test,  $p < 0.05$ ).

could help to prevent physiological drought (Rozema and Schat, 2013) and reduce ion toxicity (Yun and Shabala, 2020). Reduction of plant growth above optimal salinity ( $\geq 300$  mmol L<sup>-1</sup> NaCl; although growth was still similar to non-saline condition) possibly a strategy of the plant to survive by investing more energy in salt resistance mechanisms like ion transport, osmolyte synthesis, signaling cascade etc. Previously, Rasool et al. (2019) reported that increased respiration of *H. perfoliata* helps plants to deal with high salinity. In general, succulent salt

marsh species (*Arthrocnemum macrostachyum*, Redondo-Gómez et al., 2010; *Suaeda fruticosa*, Hameed et al., 2013; *Salicornia fruticosa*, Marco et al., 2019) restrict their growth at high salinity that referred to as a “life insurance” policy by Pujol et al. (2001) to better deal the osmotic and ionic constraints of high salinity.

Among different markers of osmotic stress decrease in leaf osmotic potential ( $\Psi_s$ ) indicates osmotic adjustment under saline conditions (Munns and Tester, 2008). Succulent plants could maintain low tissue  $\Psi_s$  to ensure

**Table** Selective absorption (SA) and selective transport (ST) of  $K^+$ ,  $Ca^{++}$ , and  $Mg^{++}$  over  $Na^+$  in *H. perfoliata* grown under different NaCl (0, 150, 300, 600 mmol  $L^{-1}$ ) treatments. Values are mean  $\pm$  standard error. Values with different letters are significantly different from each other (Bonferroni test,  $p < 0.05$ ).

Selective Absorption			
NaCl	$K^+/Na^+$	$Ca^{++}/Na^+$	$Mg^{++}/Na^+$
0	01.49 $\pm$ 0.35b	03.37 $\pm$ 0.19c	05.52 $\pm$ 0.85c
150	10.10 $\pm$ 3.14a	31.43 $\pm$ 13.97ab	24.27 $\pm$ 4.16b
300	10.24 $\pm$ 2.91a	20.24 $\pm$ 3.67b	26.99 $\pm$ 6.18b
600	12.83 $\pm$ 0.04a	36.53 $\pm$ 1.06a	42.50 $\pm$ 5.69a
Selective Transport			
NaCl	$K^+/Na^+$	$Ca^{++}/Na^+$	$Mg^{++}/Na^+$
0	0.44 $\pm$ 0.13ab	0.04 $\pm$ 0.01a	0.26 $\pm$ 0.07c
150	0.35 $\pm$ 0.12b	0.03 $\pm$ 0.02a	0.50 $\pm$ 0.10b
300	0.69 $\pm$ 0.24a	0.04 $\pm$ 0.00a	0.47 $\pm$ 0.01b
600	0.59 $\pm$ 0.01a	0.03 $\pm$ 0.00a	0.99 $\pm$ 0.08a

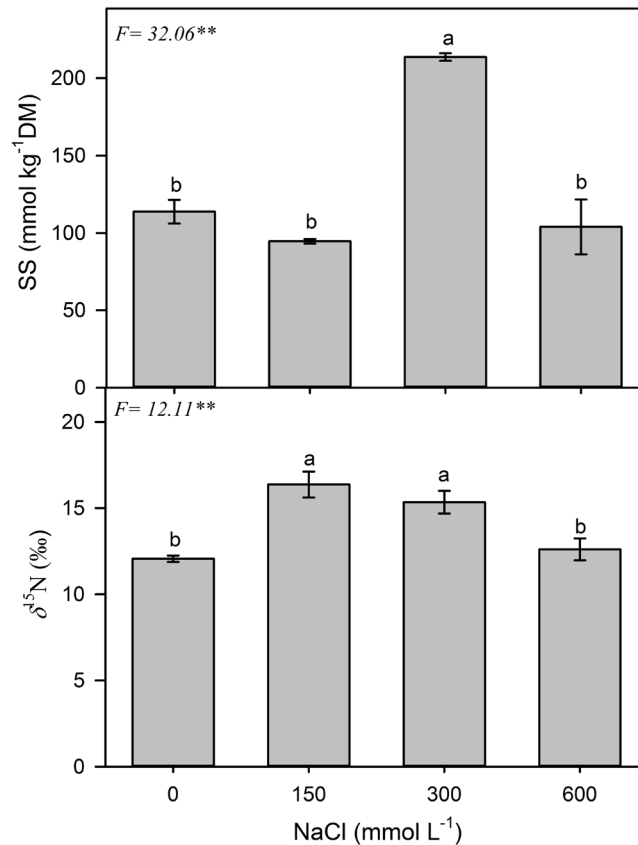
water uptake under salinity (Hameed et al., 2012). For example, *Sarcocornia quinqueflora* shoot osmotic potential was gradually decreased with increasing salinity from 0–1000 mmol  $L^{-1}$  NaCl (Ahmed et al., 2021). In the present study, *H. perfoliata* also maintained low  $\Psi_s$  in up to 300 mmol  $L^{-1}$  NaCl which was linked with sustained cell turgidity (as reflected by unchanged RWC; data not shown) for osmotic adjustment. Moreover, this unchanged leaf  $\Psi_s$  might result from increased nitrogenous based osmotic adjustment (as considerable increase in  $\delta^{15}N$ ) at 150 mmol  $L^{-1}$  NaCl, while due to nitrogenous compounds and soluble sugar accumulation in plant of 300 mmol  $L^{-1}$  NaCl. Studies on other Amaranthaceae halophytes such as *S. europaea* have shown high accumulation of many other organic solutes such as glycinebetain in response to increasing salinity (Moghaieb et al., 2004).

Decrease in succulence with rapid lowering of  $\Psi_s$  at 600 mmol  $L^{-1}$  NaCl treatment were indication of osmotic stress that is in agreement with high WD and MDA. Maintained shoot turgor in up to  $< 300$  mmol  $L^{-1}$  NaCl could be the reason of high  $CO_2$  fixation ability and improved growth, while reduced water relation attributes at 600 mmol  $L^{-1}$  NaCl led to reduced transpiration,  $CO_2$  assimilation rates, and finally growth (Rasool et al., 2019). Considerable increase in shoot  $\Psi_s$  of salinity treated plants hints at higher intake of inorganic solutes, particularly  $Na^+$  contributes about 40% of osmotic potential (data not shown). Similar results were reported for *Halosarcia pergranulata* (Short and Colmer, 1999) and *Salicornia dolichostachya* (Katschnig et al., 2013). The major contribution of inorganic solutes ( $Na^+$  and  $K^+$  was approx. 50%) in  $\Psi_s$  is a common characteristic

of eu-halophytes, which may be accounted for by low metabolic cost for osmolyte production (Munns, 2011). Shabala and Mackay (2011) also previously reported that vacuolar  $Na^+$  sequestration contributes  $\sim 50\%$  of the sap osmotic potential. Moreover, the osmotic contribution of anions is proportionately considered equivalent to cations (Munns and Tester, 2008).

Exclusion of toxic ions such as  $Na^+$  and  $Cl^-$  from cell cytoplasm and their compartmentalization are the keys for salt stress tolerance in plants (Munns and Tester, 2008). Generally, marsh halophytes accumulate more  $Na^+$  in shoots as compared to root (Flower et al., 2019; Tran et al., 2020) such as *Arthrocnemum macrostachyum*, *Salicornia brachiata* and *Sarcocornia quinqueflora* (Redondo-Gómez et al., 2010; Jacob et al., 2020; Ahmed et al., 2021). Similarly in *H. perfoliata*,  $Na^+$  was  $> 3$  fold higher in shoot of salinity treated plant in comparisons of root tissue while shoot to root ratio of  $Na^+$  content increased at high salinity. In this study, control plants also had a high amount of  $Na^+$  possibly due to the long initial culture period (18 months) because of the slow growth of *H. perfoliata*, during which plants were irrigated with half strength Hoagland solution prepared in tap-water that contains  $\sim 26$  mmol  $L^{-1}$   $Na^+$  (Ahmed et al., 2013). Being a salt accumulator, high  $Na^+$  in control plants of *H. perfoliata* is hence possible. High shoot  $Na^+$  in marsh plants including our test species *H. perfoliata* could act as cheap osmoticum which helps in water uptake (Flowers et al., 2019; Tran et al., 2020). According to Almeida et al. (2017) and Köster et al. (2018), high  $Na^+$  uptake by non-selective cation channels could decrease the passive nutrients ( $K^+$ ,  $Ca^{++}$ ,  $Mg^{++}$ ) transport which may





**Figure 5.** Soluble sugars (SS) and nitrogen isotope ( $\delta^{15}\text{N}$ ) content in shoots of *H. perfoliata* across different NaCl (0, 150, 300, 600 mmol L<sup>-1</sup>) treatments. Bars are mean  $\pm$  standard error. Bars with different letters are significantly different from each other (Bonferroni test,  $p < 0.05$ ).

result in disturbance of ion homeostasis. Under salinity, reduction of K<sup>+</sup> uptake from soil and replacement of K<sup>+</sup> by Na<sup>+</sup> in different plant parts is harmful for plant growth and metabolism. However, unlike sensitive crops halophytes can maintain K<sup>+</sup> under saline conditions. In this study, *H. perfoliata* also maintained an adequate amount of K<sup>+</sup> concentration under salinity that shows high selectivity of K<sup>+</sup> over the Na<sup>+</sup> (high SA). Similarly, many other members of Salicornioideae, for example *Tecticornia pergranulata*, *T. indica* and *Sarcocornia quinqueflora* (Colmer et al., 2009; English and Colmer, 2013; Ahmed et al., 2021), could also maintain K<sup>+</sup> ion homeostasis in tissues under salinity (Benito et al., 2014; Munns and Tester, 2008; Hussin et al., 2013). Unaltered K<sup>+</sup> homeostasis and shoot versus root K<sup>+</sup> ratio in salt tolerant species showed that shoot K<sup>+</sup> transport was not affected by high salinity (Katschnig et al., 2013; Yun and Shabala, 2020). Ca<sup>++</sup> is extensively reported in the literature as a signaling molecule (Spalding and Harper, 2011), and regulates Na<sup>+</sup> flux (Ji et al., 2013). In addition, Ca<sup>++</sup> contributes to controlling the

enzyme activity (Plieth, 2005) and the integrity of both cell wall and plant membranes (either cell membrane or organelles) (Marschner, 1995). In our experiment, plant Ca<sup>++</sup> was unaffected with salinity increments, as previously reported in *Tecticornia* species (English and Colmer, 2013) while decreased in *Halocnemum strobilaceum* with increasing salinity (Pujol et al., 2001). Magnesium (Mg<sup>++</sup>) is reported to play an important role for the activation of many enzymes, ribosome aggregation, and synthesis of photosynthesis pigments (Shaul, 2002). In *H. perfoliata*, a negative relationship between shoot and root Mg<sup>++</sup> concentration under salinity is representing the plant strategy to increase transport of Mg<sup>++</sup> towards photosynthetically active tissues. Rabhi et al. (2018) reported that salinity enhances the selective transport of Mg<sup>++</sup> with the help of both high and low-affinity transport systems. Similar results were also found in the case of *Salicornia europaea* (McNulty, 1985); *Limonium stocksii* (Zia et al., 2008) and *Salicornia brachiata* (Jacob et al., 2020). Increased shoot Mg<sup>++</sup> in *H. perfoliata* at high

NaCl treatment might be a consequence of chlorophyll degradation. Plants maintain nutrient status under salinity by increasing selectivity of  $\text{Ca}^{++}$  (Swarbreck et al., 2013) and  $\text{Mg}^{++}$  (Chen et al., 2009). *Halopeplis perfoliata* maintained  $\text{K}^+$ ,  $\text{Ca}^{++}$ , and  $\text{Mg}^{++}$  concentrations in shoot tissues at high salinity that reflects no nutrient deficiency, but the lower plant growth could be a strategy of the plant to balance ion homeostasis (Ning et al., 2015).

Soil with high  $\text{Cl}^-$  content reduces  $\text{NO}_3^-$  uptake resulting in decreased nitrogen metabolism in plants (Guo et al., 2017). In our study, a considerable increase in  $\delta^{15}\text{N}$  up to 300  $\text{mmol L}^{-1}$  NaCl is in agreement with the high plant growth at 150  $\text{mmol L}^{-1}$  NaCl and seems to be related to the synthesis of nitrogen-based osmolytes at 300  $\text{mmol L}^{-1}$  NaCl, whereas the trend of  $\delta^{15}\text{N}$  at 600  $\text{mmol L}^{-1}$  NaCl, indicates the hindrance in nitrogen transport or/and metabolism for better growth under high salinity as previously reported in case of *Suaeda salsa* (Song et al., 2009). These data, thus, hint that, at high salinity, our test species may be investing more energy towards  $\text{Na}^+$

compartmentalization for survival, rather than towards N-metabolism for active growth.

In conclusion, our results indicate that improved growth of *H. perfoliata* at 150  $\text{mmol L}^{-1}$  NaCl was linked to increased succulence and N metabolism, reduced  $\text{Na}^+$  influx while maintaining nutrient homeostasis, whereas growth is restricted at 600  $\text{mmol L}^{-1}$  NaCl due to more energy invested in shoot  $\text{Na}^+$  accumulation for osmotic adjustment and increased nutrient selective absorption. These findings, thus, provide physio-chemical basis for the ecological distribution of *H. perfoliata* in coastal areas inundated with high seawater salinity.

### Conflict of interest

The authors declare that they have no conflict of interest.

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