Scientia Horticulturae 280 (2021) 109935

Contents lists available at ScienceDirect

Scientia Horticulturae

journal homepage: www.elsevier.com/locate/scihorti

Effect of sea water substitution on growth, physiological and biochemical processes of coconut (*Cocos nucifera* L.) seedlings—A hydroponic study

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ARTICLE INFO

Keywords:
Coconut seedlings
K^+/Na^+ratio
Salinity
Sea level rise
Growth
Photosynthesis

ABSTRACT

Coconut is grown along the coasts and islands that are vulnerable to climate change-induced sea level rise. Though coconut is considered moderately salt tolerant, our understanding on the growth and physiological response to sea water, either inundation or subsurface water contamination, is very limited. This understanding will enable to effectively manage coconut in coastal systems under future climatic scenarios. In this study, ten month old hydroponically grown coconut seedlings were subjected to 0, 10, 25, 50, 75 and 100 % of sea water substitution (SWS), equivalent to 2.17, 8.32, 16.32, 30.03, 42.14 and 53.69 dS m^{-1} EC, respectively. Substituting Hoagland solution in hydroponic system by sea water of increasing concentration (>50 % SWS) significantly changed physiological processes; Fv/Fm decreased and r_s increased as early as 7 and 18 days after treatment imposition (DAT), respectively which led to significant decline in leaf area and root length expansion as early as 24 DAT. At 25 % SWS, root system (root length and root biomass) was stable but the aerial part biomass was declined by 47 %. On the other hand plant height, leaf area, collar girth and biomass accumulation of seedlings under 10 % SWS (8.32 EC) was on par with the control plants suggesting coconut seedlings could tolerate 10 % SWS. Though, P_N declined by 19 % and 43 % at 10 % and 25 % SWS, respectively and a similar decline in g_s without a concomitant change in leaf water potential suggested that root-generated signals regulated the stomatal movement in coconut under salinity. Still the biomass accumulation at 10 % SWS was not affected by decline in P_N. Under increasing sea water treatments, most of the Na^+ absorbed was compartmentalized in root and shoot, while leaf had more accumulation of K^+, that ensured high K^+/Na^+ ratio in the leaves which is an important salinity tolerant mechanism observed in coconut. The leaf Cl^- content also had strong negative correlation with [P_N] (r=-0.873) and biomass (r=-0.833), therefore in addition to K^+ and Na^+ homeostasis, the level of tolerance to the increased Cl^- content in the leaves may also play an important role in salinity tolerance of coconut. This understanding will help in making appropriate strategies for managing coconut grown at coastal systems in the face of sea level rise under climate change.

1. Introduction

Sea level rise has been a major problem as a consequence of climate change induced global warming. Process-based models project, global mean sea level to rise by 0.32 to 0.63 m for representative concentration pathway (RCP) 4.5 and 0.45 to 0.82 m for RCP 8.5 by the end of the century (Church et al., 2013). According to Stocker et al. (2013) a rise of 0.66 m sea level can inundate low lying wetlands, erode sea shores, increase salinity, rise coastal salt water tables, and exacerbate coastal flooding and also salt water intrusion into estuaries and aquifer. The majority of the vulnerable coastal regions are within 1 m elevation of sea level (Lazrus, 2012; Williams, 2013). Farmers in some of the Polynesian Tuamoto Archipelago point out increasingly frequent phenomena of high tidal swell and salt water intrusions from the ocean of atolls. This contributes to high salinization of soils and the fresh water lens on atolls, causing a decline in coconut production (Prades and Ollivier, 2013).

Abbreviations: CI, chlorophyll index; CUPRAC, Cupric ion reducing antioxidant capacity; EC, electrical conductivity; FRAP, ferric reducing antioxidant power; Fv/Fm, maximum quantum yield of Photo System II; [g_s], stomatal conductance; rs, stomatal resistance; MGD, Malayan green dwarf; PAR, photosynthetically active radiation; [P_N], net photosynthetic rate; PPO, polyphenol oxidase; RCP, Representative Concentration Pathway; ROS, reactive oxygen species; SWS, sea- water substitution; SOD, super-oxide dismutase; E, transpiration rate.

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https://doi.org/10.1016/j.scienta.2021.109935
Received 9 July 2020; Received in revised form 8 January 2021; Accepted 10 January 2021
Available online 25 January 2021

Among the 10 insular biodiversity hotspots (Bellard et al., 2014), 8 are spread over 3927 islands with submergence risk ranging from 231 to 700 (Bourdeix and Prades, 2017). In India the coconut growing east coast regions, exhibited a sea level rise of 1.92 mm year^{-1} while in Arabian Sea in the west coast the rise is 1.72 mm year^{-1}, which are in accordance with the global estimate of 1−2 mm year^{-1} (Chowdhury and Behera, 2015). The salinity level of sea water is about 3.5 % (35 g/L, 599 mM) predominantly comprising dissolved salts of Cl^-, Na^+, SO_4 - etc. having a conductivity of about 53 dS m^{-1}. Coconut is considered as a moderately salt tolerant (Marinho et al., 2006; Neto et al., 2007; Lima et al., 2017), however little is known how coconut would respond to sudden exposure to sea water, either through inundation or subsurface contamination, because of sea level rise. Therefore examining tolerance and responses of coconut to sudden exposure to sea water is vital for devising appropriate strategies for managing coconut grown at coastal systems in the face of sea level rise under climate change.

Based on salinity tolerance plants are either classified as halophytes, which can complete their life cycle under high salinity (200 mM of NaCl) (Sairam and Tyagi, 2004) and glycophytes, which cannot grow and survive under high salinity (Kozlowski, 1997; Parida and Das, 2005a,b). From the previous studies it is understood that coconut is a salt-tolerant glycophyte, with small reductions in growth and yield when irrigated with water of an electrical conductivity up to 5.0 dS m^{-1} (Marinho et al., 2006; Neto et al., 2007; Lima et al., 2017). In general coastal species are significantly more salt tolerant than those from the inland wet forests (De Sedas et al., 2019), although the mechanisms behind this tolerance remains unknown.

In most plants, salinity curtails growth via water stress and the resulting cell injury caused by the inability of vacuoles to sequester Na^+ and Cl^- (Blum, 2011). The increasing salinity affects the process of photosynthesis due to stomatal closure and/or modulations in the photochemical and biochemical processes (Praxedes et al., 2010). This results in stunted plant growth and leaf senescence (Munns and Tester, 2008). While studies have shown that some plant responses to salinity stress mimic the responses to water deficit stress (Fricke and Peters, 2002), homeostasis under salt stress depends on the ability to manage high ion concentrations (Flowers et al., 2015). Therefore, preventing Na^+ uptake and entry into the transpiration stream is the first mechanism employed to avoid salinity stress (Munns and Tester, 2008). However, as salinity increases, the ability to restrict Na^+ uptake decreases, causing increased cytosolic concentrations of Na^+ (Møller et al., 2009). Increased cytosolic Na^+ has a negative impact on osmotic balance similar to the effect caused by K^+ (Maathuis et al., 1997; Shabala and Cuin, 2008), suggesting that plant salinity tolerance is intrinsically associated with the ability to use K^+ to maintain Na^+/K^+ ratio. Yet, salinity tolerance among the glycophytes cannot be solely determined by plants ability to maintain Na^+/K^+ ratio given the inherent toxicity of Cl^- as entrance of this ion appears to be less regulated than Na^+ in some plants (Díaz-López et al., 2012). Lima et al. (2017) demonstrated that accumulation of organic solutes (carbohydrates and soluble amino-N) in roots could play a role in salt tolerance of coconut. Nevertheless, the various adaptive mechanisms of plants towards salinity stress including osmotic adjustment-effectively accumulating compatible low molecular weight solutes, to reduce the osmotic potential, enzymatic and non-enzymatic anti-oxidants scavenging reactive oxygen species (ROS), maintenance of cell turgor etc are poorly understood in coconut.

Few studies conducted to understand the impact of salinity in coconut are either on soil with fixed salinity levels (da Silva et al., 2017) or with the application of common salt (Remison and Iremiren, 1990). However, majority of the work on developing selection criteria for improved salt tolerance (Munns et al., 2002: Genc et al., 2007) and investigating the physiological and morphological effects of salt stress, etc (Xiao-Hua et al., 2009; Mahjoor et al., 2016; Rahneshan et al., 2018; Mousavi et al., 2019) have been performed using solution culture, either in hydroponic or supported hydroponic systems. Hydroponic system facilitates the physiological and nutrient kinetic studies and bypasses the spatial heterogeneity of chemical and physical soil characteristics (Rivelli et al., 2010). In this study we examined the growth, physiological and biochemical response of hydroponically grown seedlings of dwarf coconut variety to sea water salinity and made an attempt to understand the mechanism of tolerance.

2. Material and methods

2.1. Study site

Experiment was conducted in a shade net facility located at the farm of ICAR-Central Plantation Crops Research Institute (ICAR-CPCRI), Kasaragod, Kerala, India. CPCRI is located at 12° 18′ N latitude and 75 °E longitude, and at an altitude of 10.7 m above mean sea level. Plants were grown under a green shade net roof (HDPE mesh of 40–50% shading) which prevented the complete sunlight falling on the young coconut seedlings to prevent them from scorching (Fig. 1). During noon the photosynthetically active radiation (PAR) of the site was around 2000 μ mole m^{-2} s^{-1} while it was around 1200 μ mole m^{-2} s^{-1} under the shade net. Inside the shade net, the plants were grown under ambient condition with T_{max} (maximum temperature) of 34 °C, T_{min} (minimum temperature) of 24 °C, and relative humidity of 50–60%.

2.2. Plant material

A popularly cultivated dwarf coconut variety Malayan green dwarf (MGD) was used for this study. It possess traits for high yield and resistance to root (wilt) disease, it has already been released for cultivation in the root (wilt) disease prevalent tracts (Thomas et al., 2015). Because of their dwarf nature they are easy to handle for the hydroponic study and hence the genotype was selected. The seedlings were raised in polythene bags at ICAR-CPCRI farm and six month old seedlings were transplanted to hydroponic system.

2.3. Growth conditions

The response of coconut seedlings to sea water concentrations was studied in a hydroponic system. Large plastic drums of 60 L capacity of height 70 cm and diameter 30 cm were used to grow the seedlings. In the top lid, at the center a hole of 15 cm diameter was bore to place the plants and little away three more small holes were made as a provision

Fig. 1. Figure showing the experimental setup for growing coconut seedlings in the hydroponic system underneath a shadenet. Plants were tied to pot lid as well as external rods for additional support. The black pipe inserted through the lid ensures continuous aeration within pots.

to tie the plants. Another smaller hole was made at the corner of lid for inserting the microcapillary pipe for aeration (Fig. 1). The outer surface of the drums was painted black in order to prevent the entry of sunlight and to avoid the growth of algae in the nutrient medium. A Hoagland's solution had the following nutrient composition of which macronutrients (in mM) were: KNO_3 (5); Ca $(NO_3)_2$. $4H_2O$ (4); $MgSO_4.7H_2O$ (1); KH_2PO_4 (2); and the micronutrients were KCl (0.05); H_3BO_3 (0.025); $MnCl_2$ (0.002); $ZnSO_4$ (0.002); $CuSO_4$ (0.0005); Na_2MoO_3 (0.0001) and Fe Na EDTA (0.064). The drums were filled with 40 L of water enriched with full strength Hoagland's nutrient solution (pH 5.8).

Six months old uniform healthy coconut seedlings were extracted from the polythene bags (on 5 July 2018), all the roots surrounding the nut were cut, washed thoroughly and inserted through the lid hole in such a way that nut was inside the water and the shoot and leaves were above the lid. Each pot contained single seedling. In order to hold the plants in erect position and to adjust proper positioning of nut and roots as with the level of water in the pot, plastic ropes inserted through the nut exocarp were pulled and fastened outside lid hole (Fig. 1). Additionally support was provided to shoots to keep them erect. A micro tube connected to each pot and immersed in water drew the air from the main line, which was connected to a 1.5 HP compressor to ensure adequate and continuous aeration to the plants. The seedlings were acclimatized to grow and stabilize in the nutrient solution for another four months.

2.4. Experimental design and salt water treatments

After four months of stabilization in hydroponics, ten month old seedlings of uniform health (both roots and above ground parts) were selected for sea water imposition. We employed randomized design with five sea water concentrations. The Hoagland solution in the pots were substituted by 10, 25, 50, 75 and 100 % sea water (SWS equivalent to 0, 4, 10, 20, 30, and 40 L sea water, respectively), while control plants (0% sea water) were grown in 40 L of one strength Hoagland solution. Sea water used in the experiment was collected from Arabian Sea in the vicinity of ICAR-CPCRI farm. Each treatment was replicated three times. The nutrient solution was renewed after every 15 days and the pH of fresh solution was maintained 5.8 by adding either sodium hydroxide (NaOH) or hydrochloric acid (HCl) as described by Kargbo et al. (2019). The experiment was continued for a period of six months and terminated on 4th, May 2019. During the course of the experiment at regular intervals growth and physiological observations were recorded. After six months of salt treatment, leaf, stem, and root samples were harvested from control and sea water-treated plants for estimation of various parameters. Leaves occupying the same position (top most fully opened) from control and sea water treated plants were used for physiological measurements and leaflets were sampled from same position for estimation of various biochemical parameters.

2.5. Growth measurements

Plant height was recorded from plant base to the highest point of the fully opened leaf. The circumference of the stem just above the attachment of nut was measured at a fixed point using measuring tape and expressed in centimeters is the collar girth. Length and width of each fresh leaf was measured to calculate the whole plant leaf area using the linear regression equations developed by Mathes et al. (1989). Fresh biomass of the whole seedling was recorded by removing the seedling from the pot and after the excess water on the roots were dried using paper towel. Leaf area, collar girth, length of longest root, fresh weight of the plant was periodically recorded starting from 24 DAT. At the end of the experiment, seedlings were separated into root, shoot and leaf oven dried at 65 °C for two days and dry weights were recorded to calculate the total dry biomass production.

2.6. Physiological measurements

Stomatal resistance (r_s) and chlorophyll fluorescence of the treatments were measured periodically from 18 and 7 DAT respectively on the adaxial surface of young fully opened leaf using a porometer (Porometer AP4, Delta-device, Cambridge, UK) and expressed as seconds per centimeter (s cm^{-1}). Chlorophyll fluorescence indices of the leaf were measured after dark-adaption for 30 min, using a chlorophyll fluorescence meter (OS30p, OptiSciences, Hudson, MH, USA). Towards the end of the experiment (160 DAT) leaf water potential (Ψ) of leaflets from topmost fully opened leaf was measured using a Scholandar Pressure Bomb (WESCOR). Leaflets from the same position were used for the measurement of process parameters *viz*. net photosynthetic rate (P_N), stomatal conductance (g_s) and transpiration (E) using a portable photosynthesis system (LI-COR 6400XT, LI-COR, Lincoln, NE, USA). Measurements were made in triplicates at a fixed light intensity of 1500 μmol m^{-2} s^{-1}. Chlorophyll content was measured by Leaf chlorophyll meter (Hebbar et al., 2016)

2.7. Biochemical analysis

Leaf samples collected (160 DAT) were immediately placed in an ice box. Leaf tissue (0.5 g) was ground in 10 ml of 80 % ethanol. The extract was subjected to 30 min of rotospin and later sonicated for 30 min. After the centrifugation at 6000 rpm for 15 min the supernatant was collected. To the pellet 5 ml 80 % alcohol was added and the extraction procedure was repeated. The pooled supernatant was evaporated at 80 °C in the water bath until a drop was left. Finally the extract was dissolved at 80 °C and made up with 10 ml of distilled water. The extract was stored in deep freezer (−20 °C) for the analysis of biochemical parameters.

Total soluble sugar content in the extract was determined using phenol-sulphuric acid method (DuBois et al., 1956). The reducing sugar content was determined by Nelson-Somogyi's method using arsenomolybdate reagent (Somogyi, 1952). Results are expressed as grams of glucose equivalents per 100 mL using standard curve. Free amino acids were estimated using ninhydrin method (Moore and stein, 1954) and measured at 570 nm using the standard leucine (1 mg/1 mL).Total phenol content was estimated by Folin-Ciocalteu's method (Bray and Thorpe, 1954). The blue color complex developed was quantified by visible-light spectrophotometer (Shimadzu UV 160A). The polyphenols content of the samples were expressed as gallic acid equivalent (GAE) g^{-1} FW. Cupric ion reducing antioxidant capacity (CUPRAC) (Apak et al., 2008) and ferric reducing antioxidant power (FRAP) (Benzie and Strain, 1996) was measured in the extracts and expressed as μmol trolox equivalent (TE) g^{-1} FW. The leaf membrane stability was measured following the protocol described by Huang et al. (2006).

2.8. Analysis of leaf enzymatic anti-oxidants

Enzyme extract from the leaf tissue was prepared in sodium phosphate buffer (0.1 M, pH 7.6) by following the method standardized for coconut leaf tissue (Chempakam et al., 1993). Briefly, 0.5 g of leaf tissue was ground in 15 mL sodium phosphate buffer, along with PVPP and sand. The extract was centrifuged at 4 °C for 20 min and the supernatant obtained was pooled for the assay of super oxide dismutase (SOD) (Beauchamp and Fridovich, 1971), polyphenoloxidase (Kar and Mishra, 1976). The soluble protein content of the leaf samples was measured according to Bradford (1976) using bovine serum albumin as a standard. One unit of SOD activity was defined as quantum of enzyme required to cause 50 % inhibition of photoreduction rate. Similarly, PPO activity was measured based on the increase in absorbance due to the conversion of pyragallol to o-quinone and one unit of PPO activity was defined as the amount of enzyme that produces 1 micromole of o-dopaquinone per minute

2.9. Nutrient analysis

Sea water and hydroponics water samples were collected in polyethylene bottles after thoroughly rinsing the bottles with the respective samples. Samples were stored in a refrigerator at 4 °C until analysis. Sea water was analyzed for the chemical composition following standard methods (APHA, 2005). The pH and EC were determined using a Eutech multiparameter model PC2700. The Na^+ and K^+ contents were determined using flame photometer (Elico CL378) (Williams and Twine, 1960), Cl^- content was estimated by titrating with 0.1 N $AgNO_3$. Ca^{2+} and Mg^{2+} contents were analyzed titrimetrically using EDTA standard solution. The bicarbonate (HCO_3^-) content was estimated by titrating against H_2SO_4 standard solution, SO_4^{2-} content of sea water was analyzed spectrometrically following turbidimetry method (APHA, 2005, 4500-SO_4^{2-}, E. Turbidimetric method). Determination of PO_4^{3-} content was carried out following ascorbic acid spectrometric method (APHA, 2005, 4500-P, E. Ascorbic Acid Method) and boron was determined spectrophotometrically with azomethane H reagent using UV Visible Spectrophotometer (Shimadzu UV 160A). Micronutrients *viz.*, Fe^{2+}, Mn^{2+}, Zn^{2+} and Cu^+ contents were determined using atomic absorption spectrophotometer (ICE 3300, Thermo Fisher Scientific). Hydroponics water samples of all the treatments were analyzed for pH and EC.

Root, shoot and leaf samples of coconut seedlings from each treatment were collected separately, washed in tap water and then cleaned with double distilled water. Thereafter, samples were initially air dried and then oven dried at 70 °C for 72 h in a hot air oven. Oven dried samples were powdered and homogenized. Ground plant material was wet digested (Piper, 1966) by di-acid mixture of HNO_3 and $HClO_4$ in the ratio of 9:4 respectively. The contents of K^+ and Na^+ in the digested extracts were determined (Williams and Twine, 1960) using flame photometer (Elico CL378). Ca^{2+} and Mg^{2+} were determined by versenate titration method (Diehl et al., 1950), Chloride was determined by Mohr's method, oven dried ground samples were extracted with Ca $(NO_3)_2$ in l:100 ratio and titrated with $AgNO_3$ (Silva et al., 1998).

2.10. Statistical analysis

Each sea water treatment had three replications and the growth and physiological observations were repeated over a period of time. The data was analyzed using one way analysis of variance (ANOVA) of repeated measures and the treatment means were compared by Duncan's multiple – range test ($P \leq 0.05$) using the statistical software SAS 9.3.

3. Results

3.1. Physical and chemical properties of sea water and nutrient solutions

The solute water fraction of the sea water collected from the Arabian sea used in the experiment is rich in Cl^- followed by Na^+, SO_4^{2-} and Mg^{2+} ions and are well within the range of the composition of reference sea water (Table 1). The pH of sea water is slightly alkaline (7.63) and has EC of 53.78 dS m^{-1}. The nutrient solution in the control (0% SWS) pot had a pH of 5.76 and EC 2.17 dS m^{-1} and both of these parameters were found to increase due to sea water substitution (Table 2). At 10, 25, 50, and 75 % SWS the EC increased to 8.32, 16.32, 30.03, 42.14 dS m^{-1}, respectively. The pH of the freshly renewed solution in all the pots was adjusted to 5.8 using small quantity of 1 N HCl.

3.2. Growth response

Plant height, collar girth, leaf area, root length and fresh weight of ten month old hydroponically grown coconut seedlings at commencement of treatment imposition was 170 cm, 29 cm, 2.6 m^2, 19 cm, 3.3 kg $plant^{-1}$ (Figs. 3–6), respectively. The corresponding values at the end of the experiment was 222 cm, 44.16 cm, 5.52 m2 (Table 3), 46 cm (Fig. 5) and 7.28 kg $plant^{-1}$ (Fig. 6) respectively. Increasing sea water substitution significantly declined the plant height ($p = .009$), leaf area ($p < .0001$) collar girth ($p = .0016$) and root length and fresh weight of the seedlings (Table 3 and Figs. 3–6). SWS of >50 % significantly reduced the leaf area (Fig. 3) and root length (Fig. 5) at the earliest recorded observation of 24 days after treatment imposition (DAT). At 50 % SWS significant decline in leaf area and root length was recorded 45 and 57 DAT respectively. On the other hand at 25 % SWS, there was no significant effect on root length while leaf area was significantly low 85 DAT. Collar girth and fresh weight declined significantly from control 24 DAT with 100 % SWS, 45 DAT with 75 % SWS and 85 DAT with 25 % SWS. All the above parameters at 10 % SWS were on par with control. Unlike the aerial parts, the effect of sea water treatment was relatively less pronounced on the roots. Root length elongation rate at 25 % SWS was comparable with that of control and 10 % SWS (Fig. 5), as a result there were no significant differences in the root dry biomass between the control, 10 and 25 % SWS 160 DAT (Table 3). However, seedlings grown at and above 50 % SWS, showed a significant decline in root weight ($p = .006$). The whole plant fresh weight had increased to around 7.5 kg in control and in 10 % SWS from the initial 3.5 kg (Fig. 6). During the same period seedlings grown at 25 % SWS showed an increase of whole plant fresh weight to 4.8 kg and at 50 % SWS registered no increase. Consequently, seedlings at 25 % SWS, produced 47 % less dry biomass from the control (1.22 kg). The fraction of biomass in root: shoot: leaf was 0.07: 0.46: 0.46 for control and 10 % SWS plants while it was 0.11: 0.45: 0.43, respectively at 25 % SWS (Table 3).

Table 1
Weight fraction of solutes (g kg^{-1}) of sea water sample from Arabian Sea used in this experiment in comparison with the composition of reference sea water.

Solutes	Sample values	Reference values (Millero et al., 2008)
Cl^-	19.26	19.35
Na^+	10.63	10.78
SO_4^{2-}	2.80	2.71
Mg^{2+}	1.34	1.28
Ca^{2+}	0.40	0.41
K^+	0.39	0.40
HCO_3^-	0.15	0.10
$B(OH)4^-$	0.004	0.008
Fe^-	0.002	0.0035
Others		
P	0.080	
Zn	0.009	
Mn	0.002	
Cu	0.004	

Table 2
The pH and electrical conductivity (EC) of sea water and different sea water treatments used in the experiment.

Treatments	pH	EC (dS m^{-1})
Control	5.76	2.17
10 % SWS	6.02	8.32
25 % SWS	6.12	16.32
50 % SWS	6.35	30.03
75 % SWS	6.56	42.14
100 % SWS	6.65	53.69
Sea water	7.63	53.78

3.3. Physiological response

Sudden, significant changes in physiological processes like r_s and Fv/Fm were recorded in response to increasing SWS. Stomatal resistance (r_s) on 18 DAT was 1.86, 3.71, 7.89, 67.33, 121.66 144.33 cm s^{-1} with 0, 10, 25, 50, 75 and 100 % SWS, respectively (Fig. 7). The increase was significant at 50, 75 and 100 % SWS from control. The initial r_s value remained stable with 50 % SWS but went out of measuring range of instrument with 75 and 100 % SWS at 105 and 71 DAT respectively. Throughout the measurements r_s of 10 % SWS was on par with control

Table 3
Growth parameters (plant height, collar girth, leaf area) and dry biomass of root, shoot, leaf of sixteen months old coconut seedlings subjected to the increasing level of sea water. Data are mean value of 3 replicates. Mean with same letter represent non significance while different letter represent significance. Significance level of each factor is indicated by p- values and standard error of mean (SEm).

Treatments	Plant height (cm)	Leaf area (m^2)	Collar girth (cm)	Dry weight (g $plant^{-1}$)			
				Root	Shoot	Leaf	Total
Control	222^{a}	5.52^{ab}	44.16^{a}	93.30^{a}	569.00^{a}	561.00^{a}	1223.30^{a}
10 % SWS	215^{ab}	5.11^{ab}	44.33^{a}	82.30^{a}	506.60^{a}	533.00^{a}	1122.00^{a}
25 % SWS	195^{cb}	3.44^{cb}	37.73^{ab}	79.60^{a}	290.10^{b}	288.10^{b}	658.50^{b}
50 % SWS	180^{cb}	2.04^{cd}	31.06^{bc}	14.00^{b}	162.00^{bc}	164.20^{b}	340.20^{bc}
75 % SWS	165^{c}	0.37^{ed}	24.00^{c}	14.00^{b}	73.30^{c}	161.50^{b}	248.90^{c}
100 % SWS	160^{c}	0.01^{e}	29.33^{bc}	7.30^{b}	77.30^{c}	171.00^{b}	255.60^{c}
SEm	20	1.65	7.55	5.10	12.02	30.00	40.00
p- value	0.009	<.0001	0.0016	0.0069	0.0003	<.0001	<.0001

while at 25 % SWS the increase was significant beyond 83 DAT. Fv/Fm measured at 7 DAT was significantly low at 75 (0.572) and 100 % (0.367) SWS compared to control (0.825), which continued to remain low throughout the measurements (Fig. 8). At 50 % SWS it was significantly low from 28 DAT. At 10 and 25 % SWS it was on par with control.

Net photosynthetic rate (P_N) measured towards the end of the experiment was 6.42 µmole m^{-2} s^{-1} for control plant which declined significantly ($p < .0001$) to 5.18 and 3.65 at 10 % and 25 % SWS, respectively corresponding to 19 % and 43 % decline. At 50 % SWS, P_N further reduced to 1.58, a decline of 75 % from the control seedlings. Similar trend of drastic decline with the increase in SWS was observed both in stomatal conductance (g_s) and transpiration rate (E) (Table 4). Interestingly, the leaf water potential did not decline significantly up to 10 and 25 % SWS, however at 50 % SWS and beyond leaf water potential showed a significant decrease ($p = .009$) (Table 4). Chlorophyll index (CI) did not decline significantly up to 25 % SWS. Membrane stability index showed significant ($p < .0001$) decline at 25 % SWS and beyond.

3.4. Biochemical response

The concentration of total sugars and reducing sugars in the leaves of coconut seedlings though increased with increasing SWS they were not significant (Table 5). Free amino acid which was 2.81 µg g^{-1} FW in control increased significantly ($p = .0005$) to 5.33 µg g^{-1} FW at 25 % SWS and beyond. Similarly, total polyphenol content showed significant ($p = .014$) increase at 75 % SWS (84.71) and 100 % SWS (93.69) from control (55.73 µg GAE g^{-1} FW). With the increase in total polyphenol content, salinity treatments have caused concomitant increase in antioxidant potential quantified by CUPRAC ($p = .007$) at 50 % SWS and FRAP ($p = .067$) at 25 % SWS. Significant increase in free radical scavenging enzyme activities of SOD ($p = .0006$) and PPO ($p = .003$) was observed at 25 % SWS and beyond (Table 5). The control plant SOD and PPO activity of 11.16 and 0.49 had increased to 16.24 and 1.57 U g^{-1} FW at 25 % SWS, respectively.

3.5. Nutrient content

The K^+ content in root, shoot and leaf was 482.05, 505.12 and 446.15 µmole g^{-1} DW, respectively (Table 6). Sea water treatment significantly declined the K^+ content of root ($p < .0001$), shoot ($p < .0001$) and leaf ($p < .0001$). At 10 % SWS, K^+ content was reduced by 57 % in root, 17 % in shoot and only 11 % in leaf compared to the control. On the other hand there was significant increase in Na^+ accumulation, 72 % in root ($p < .0001$), 93 % in shoot ($p < .0001$) and only 16 % in leaf ($p < .0001$) at 10 % SWS from the control content of 465, 582 and 373 µ mole g^{-1} DW respectively. With the increasing sea water substitution there was significant and steep decline of K^+ content in root and shoot while the decrease was marginal in leaf and it was vice versa for Na^+ content. Consequently, the K^+/Na^+ ratio was high in leaf in all the sea water treatments followed by shoot and was the least in roots (Fig. 9). Ca^{2+}, Mg^{2+} and Cl^- content of the control leaves was 106.6, 138.2 and 149.8 µmole g^{-1} DW respectively. At 10 % SWS Ca^{2+} did not change significantly but Mg^{2+} decreased by 24 % while Cl^- increased by 69 %. With further increase especially beyond 50 % SWS, there was more decline in Ca^{2+} and Mg^{2+} and large increase in Cl^- content was recorded (Table 6).

3.6. Pearson's correlations

Correlation coefficients among the growth, physiological and biochemical parameters across the treatments analyzed by Pearson's correlation are listed in Table 7. Most of the parameters show significant correlations ($p \leq 0.05$) with dry biomass and photosynthesis. Dry biomass had a strong positive correlation with leaf area (0.962), P_N (0.911), leaf K^+ (0.864), Leaf Ca^{2+} (0.937), leaf Mg^{2+} (0.88) and root K^+. While the correlation was strongly negative with r_s (-0.825),

Table 4
Physiological parameters namely, net photosynthetic rate (P_N), stomatal conductance (g_s), transpiration (E), stomatal resistance (r_s), chlorophyll fluorescence (Fv/Fm), chlorophyll index (CI), leaf water potential (Ψ) and membrane stability index of sixteen months old coconut seedlings subjected to the increasing levels of sea water. Data are mean value of 3 replicates. Mean with same letter represent non significance while different letter represent significance. Significance level of each factor is indicated by p- values and standard error of mean (SEm).

Treatment	Photosynthesis (P_N) (µ mol m^{-2} s^{-1})	Stomatal conductance (g_s) (mol $m^{-2}s^{-1}$)	Transpiration (E) (m mol $m^{-2}s^{-1}$)	Stomatal resistance (r_s) (s cm^{-1})	Chlorophyll fluorescence (Fv/Fm Ratio)	Chlorophyll index (CI)	Leaf water potential Ψ (bars)	Membrane stability index (MSI)
Control	6.42^{a}	0.052^{a}	1.21^{a}	1.88^{c}	0.79^{a}	57.90^{a}	9.93^{b}	80.57^{a}
10 % SWS	5.18^{b}	0.045^{b}	0.92^{b}	5.38^{c}	0.79^{a}	58.23^{a}	10.06^{b}	78.97^{a}
25 % SWS	3.65^{c}	0.032^{b}	0.61^{c}	21.42^{c}	0.77^{ab}	57.43	10.93^{b}	74.67^{b}
50 % SWS	1.58^{d}	0.022^{c}	0.09^{d}	94.07^{b}	0.73^{ab}	49.37^{b}	13.95^{ab}	67.35^{c}
75 % SWS	0.22^{e}	0.001^{c}	0.06^{d}	121.04^{ab}	0.69^{b}	42.23^{b}	19.01^{a}	66.02^{c}
100 % SWS	0.0	0.0	0.0	134.33^{a}	0.57^{c}	36.10^{d}	18.18^{a}	62.99^{c}
SEm	0.68	0.006	0.25	67.43	0.35	4.63	5.40	3.86
p- value	<.0001	<.0001	<.0001	<.0001	0.0015	<.0001	0.0098	<.0001

Table 5
Biochemical responses of coconut seedlings to increase in sea water substitution. Various biochemical parameters such as total sugar, reducing sugar, free amino acids, total polyphenols, antioxidants and free radical scavenging enzymes are enumerated. Data are mean value of 3 replicates. NS denotes no significance at 5% level. Mean with same letter represent non significance while different letter represent significance. Significance levels of each factor are indicated by *p*- values and standard error of mean (SEm).

Treatment	Total sugar (mg g^{-1} FW)	Reducing sugar (mg g^{-1} FW)	Free amino acids (μg g^{-1} FW)	Total poly phenols (μg GAE g^{-1} FW)	Anti oxidant capacity (μmol trolox equivalent g^{-1} FW)		SOD(U g^{-1} FW)	PPO(U g^{-1} FW)
					CUPRAC	FRAP		
Control	8.66	2.04	2.81^{c}	55.73^{c}	22.00^{d}	5.83^{cd}	11.16^{c}	0.49^{b}
10 % SWS	8.83	2.89	2.54^{c}	44.82^{c}	25.75^{cd}	6.12^{c}	11.87^{c}	0.81^{b}
25 % SWS	8.88	4.00	5.33^{b}	61.48^{cb}	26.42^{cd}	8.02^{b}	16.24^{b}	1.57^{a}
50 % SWS	9.61	4.46	6.33^{ab}	65.28^{cb}	29.63^{cb}	8.04^{b}	17.40^{ab}	1.62^{a}
75 % SWS	12.03	6.73	6.00^{ab}	84.71^{ab}	33.05^{ab}	8.91^{ab}	17.56^{ab}	1.75^{a}
100 % SWS	12.02	5.88	7.73^{a}	93.69^{a}	36.58^{a}	10.17^{a}	18.86^{a}	2.16^{a}
SEm	NS	NS	1.52	16.31	4.15	2.06	15.85	0.40
p- value	2.7	2.2	0.0005	0.014	0.007	0.067	0.0006	0.003

Table 6
K and Na content (μmole g^{-1} DW) of root, shoot and leaf and Ca, Mg and Cl content (μmole g^{-1} DW) of leaf of coconut seedlings with increasing level of sea water substitution. Data are mean value of 3 replicates. Mean with same letter represent non significance while different letter represent significance. Significance level of each factor is indicated by p- values and standard error of mean (SEm).

Treatment	Root		Shoot		Leaf				
	K^+	Na^+	K^+	Na^+	K^+	Na^+	Ca^{2+}	Mg^{2+}	Cl^-
Control	482.05^{a}	465.21^{d}	505.12^{a}	582.60^{d}	446.15^{a}	373.91^{d}	106.66^{a}	138.27^{a}	149.87^{d}
10 % SWS	205.12^{b}	800.00^{c}	420.51^{b}	1126.08^{c}	397.43^{b}	434.78^{c}	106.66^{a}	105.35^{b}	252.05^{c}
25 % SWS	153.84^{c}	1147.82^{b}	312.82^{c}	1430.43^{b}	364.10^{b}	478.26^{bc}	80.00^{b}	92.18^{b}	296.33^{c}
50 % SWS	92.30^{d}	1365.21^{b}	243.58^{d}	1600.00^{ab}	300.00^{c}	526.08^{b}	53.33^{c}	85.59^{b}	313.36^{bc}
75 % SWS	58.97^{d}	1804.34^{a}	176.92^{e}	1739.13^{ac}	192.30^{d}	582.60^{a}	53.33^{c}	52.67^{c}	362.51^{b}
100 % SWS	25.64^{e}	1747.82^{a}	138.46^{e}	873.91^{c}	135.89^{e}	465.21^{c}	40.00^{c}	39.51^{c}	471.51^{a}
SEm	38.46	269.57	58.97	265.22	38.46	52.17	23.52	27.76	65.01
p-Value	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001

polyphenol (-0.803), SOD (-0.941), PPO (-0.934), leaf Cl^- (-0.833) and root Na^+ (-0.851).

4. Discussion

Coconut is largely grown in coastal belts and islands (Bourdeix and Prades, 2017; Child, 1974) which led to believe that it is salt tolerant, therefore it is less likely to be vulnerable to either sea water inundation or contamination of subsurface/underground water due to rising sea level under climate change. However, from the few of the previous studies conducted either on saline soil or with the application of species-specific saline water it is understood that coconut is a salt-tolerant glycophyte, with small reductions in growth and yield when irrigated with water of an electrical conductivity up to 5.0 dS m^{-1} (Marinho et al., 2006; Neto et al., 2007; Lima et al., 2017). Moreover, there are no studies to show how coconut would respond to either sea water inundation or exposure to the diluted form of sea water from the contaminated underground water. Already some of the islands (Bellard et al., 2014) and coastal belts (Bourdeix and Prades, 2017), where coconut is grown, are encountering the threat of sea level rise (Williams, 2013). Therefore understanding the growth response of coconut to sea water will help in developing appropriate strategies for managing coconut grown at coastal systems under changing climate conditions. In this study hydroponically grown ten month old dwarf variety of coconut, MGD (Malayan green dwarf), were exposed to increasing concentrations of sea water (0, 10, 25, 50, 75 and 100 %) for a period of six months. Growth, physiological processes, biochemical constituents and nutrient content were significantly affected with the increasing sea water treatment, the results of which are discussed in this section.

Unlike the preceding salinity tolerance experiments on coconut, this experiment was conducted in coconut seedlings, grown in a controlled hydroponics system, which eliminates the confounding effects of drought and limited nutrients. This is the first report of growing coconut seedlings in the hydroponic system and the plants responded very well, as the growth was comparable to the potted seedlings of similar age raised in soil (Hebbar et al., 2013). The sea water used in this experiment, which was collected from Arabian Sea, comprised mainly of Cl^-, Na^+, SO_4^{2-}, and Mg^{2+} etc. and their concentration was well within the range of the composition of the reference sea water (Millero et al., 2008). The pH of Hoagland solution (5.8) in hydroponic system had narrowly increased by the substitution of sea water (7.63) which was adjusted with the addition of few drops of 1 N HCl.

As our aim was to investigate the response of coconut to sea water, we chose treatments of a wider range involving very high concentration of 100 % (EC of 53.78 dS m^{-1}), followed by 75 % (42.14 dS m^{-1}), 50 % (30.03 dS m^{-1}), 25 % (16.32 dS m^{-1}), 10 (8.32 dS m^{-1}) and 0% (2.17 dS m^{-1}) substitution of Hoagland solution by sea water. One strength Hoagland's nutrient solution has EC of 2.0 dS m^{-1} (Kang and Van Iersel, 2002), but in our control pots it was 2.17, due to the possible contribution of ions from the irrigation water used for the preparation of Hoagland's nutrient solution.

Coconut seedlings are highly sensitive to sudden exposure of high concentration sea water. Both the roots and aerial parts are severely injured at and beyond 50 % SWS (Fig. 2). There was significant decline in Fv/Fm (maximum quantum yield of PSII) at 75 and 100 % SWS and significant increase in r_s at 50, 75 and 100 % SWS at the earliest observation made on 7 and 18 DAT respectively, suggesting both the non-stomatal and stomatal factors might have affected the growth in coconut under salinity (Brugnoli and Björkman, 1992). At 75 % and 100 % SWS, the stomata was completely closed at 18 DAT and measurement became out of range by 57 DAT which led to immediate senescence of lower leaves and the roots as a result both leaf area and root length were significantly low at the earliest observation on 24 DAT. The reduction in leaf area by 85 DAT became so severe that it caused the fresh weight to become less than the initial weight suggesting plant was survived by the utilization of the reserved carbohydrates (Almodares et al., 2008). At 50

Table 7
Pearson's correlation coefficients based on the growth, physiological and biochemical parameters of coconut cultivar WCT exposed to different level of sea water substitution (0, 10, 25, 50, 75 and 100 % SWS). Each value indicates the Pearson's correlation coefficient of a pair of parameters. Ht: plant height, LA: leaf area, CG: collar girth, DM: dry biomass, P_N: photosynthesis, rs: stomatal resistance, Fv/Fm: Max. quantum yield of PS II, LWP: leaf water potential, PP: total polyphenol, SOD: super oxide dismutase, PPO: polyphenol oxidase, LK: leaf K^+, LNa: leaf Na^+, LCa: leaf Ca^{2+}, LMg: leaf Mg^{2+}, LCl: leaf Cl^-, RK: root K^+, RNa: root Na^+. All the correlation values are significant at 0.05 levels, except those indicated by NS: non-significant.

	Ht	LA	CG	DM	P_N	r_s	Fv/Fm	LWP	PP	SOD	PPO	LK	LNa	LCa	LMg	LCl	RK
LA	0.885																
CG	0.821	0.917															
DM	0.893	0.962	0.92														
P_N	0.924	0.917	0.86	0.911													
r_s	−0.855	−0.884	−0.846	−0.875	−0.935												
Fv/Fm	0.695	0.736	0.56	0.668	0.735	−0.746											
LWP	−0.763	−0.722	−0.753	−0.726	−0.774	0.864	−0.547										
PP	−0.859	−0.852	−0.773	−0.803	−0.842	0.807	−0.824	0.679									
SOD	−0.86	−0.908	−0.846	−0.941	−0.932	0.845	−0.698	0.633	0.804								
PPO	−0.876	−0.897	−0.792	−0.934	−0.898	0.848	−0.66	0.715	0.769	0.944							
LK	0.889	0.919	0.793	0.864	0.94	−0.922	0.776	−0.776	−0.871	−0.851	−0.889						
LNa	−0.651	−0.604	−0.683	−0.64	−0.757	0.669	−0.29^{NS}	0.553	0.462	0.703	0.634	−0.611					
LCa	0.806	0.895	0.842	0.937	0.892	−0.882	0.667	−0.687	−0.826	−0.908	−0.891	0.87	−0.582				
LMg	0.844	0.896	0.811	0.88	0.897	−0.845	0.703	−0.808	−0.748	−0.839	−0.88^{NS}	0.903	−0.586	0.802			
LCl	−0.794	−0.846	−0.739	−0.833	−0.873	0.865	−0.718	0.728	0.74	0.86	0.922	−0.907	0.553	−0.823	−0.892		
RK	0.827	0.795	0.725	0.833	0.881	−0.753	0.58	−0.605	−0.632	−0.862	−0.888	0.816	−0.745	0.769	0.846	−0.864	
RNa	−0.856	−0.905	−0.851	−0.913	−0.947	0.903	−0.733	0.747	0.806	0.899	0.896	−0.916	0.734	−0.898	−0.901	0.862	−0.865

% SWS, despite high r_s, leaf area reduction was more gradual as a result plants could maintain their initial weight without any further increments. At lower SWS of 25 %, the fresh weight was significantly low in spite of r_s, leaf area and root expansion were on par with control till 76 DAT, 85 DAT and termination of experiment respectively. Only at 10 % SWS both the physiological and growth parameters were on par with control throughout the observation period suggesting that coconut dwarf variety MGD could tolerate salinity up to 10 % SWS equivalent to 8.32 dS m^{-1} EC which is slightly higher than the tolerance limit reported earlier 6.5 dS m^{-1} (da Silva et al., 2016) and 5.2 dS m^{-1} (Lima et al., 2017). These previous studies were conducted either in salt affected fields or potted plants or through application of saline water. Coconut under reasonable level of salinity in this case at 25 % SWS (EC 16.32 dS m^{-1}) invested higher biomass in roots which is on par with control even though the above ground biomass declined by 47 % which is in agreement with earlier findings (Lima et al., 2017), suggesting greater plasticity in the root architecture of coconut seedlings in countering the severe salinity stresses (Lamanda et al., 2008; da Silva et al., 2016, 2017; da Silva et al., 2018). This also suggest that the salinity tolerance limit of coconut might lie in between 10 and 25 % SWS.

Coconut seedlings suffered osmotic and ionic stress under high sea water concentration due to the salt accumulation outside the roots (Cl^-, Na^+, SO_4^{2-} and Mg^{2+} ions) and those accumulated inside of plant cells (especially Cl^- and Na^+), respectively. The former generally inhibits water uptake and cell expansion (Munns and Tester, 2008), however in this experiment the leaf water potential was stable up to 25 % SWS and salinity stress beyond that caused a significant decline in water potential. On the other hand the stomatal conductance, whose regulation depends on leaf water potential, significantly declined while stomatal resistance increased though was not significant (increased to 5.4 s cm^{-1} as against 1.88 in control at 10 % SWS), suggested that the salinity stress induced signals generated in roots (could be either chemicals like ABA or electrical potential) might have regulated the stomatal movement in coconut without declining the leaf water potential (Zhang and Davies, 1991; Hebbar et al., 1994). It is also apparent that the stomatal closure prevented the transport of water vapor and CO_2, and thus photosynthesis and transpiration decreased significantly. [P_N] decreased by 19 and 43 % at 10 % SWS and 25 % SWS, respectively. Even though, strong correlation existed between [P_N] and biomass (r = 0.911), it did not influence the biomass production at 10 % SWS as the reduction was not significant. Chlorophyll index (CI) a measure of chlorophyll content and chlorophyll fluorescence did not vary significantly up to 25 % SWS suggesting the maximum quantum yield of PSII (Fv/Fm) was not significantly affected. It was deduced that photochemical and biochemical processes of photosynthesis, leaf area reduction, stomatal closure and loss of chlorophyll are attributed to the decreased biomass or carbon assimilation capacity of the plants (Taiz et al., 2015; De Medeiros et al., 2018).

The greatest accumulation of potentially toxic ions (particularly Na^+ and Cl^-) in the leaf tissues of glycophytes is correlated with the sensitivity to salt stress (Tester and Davenport, 2003; Trindade et al., 2006). In the present study, however, there was a high accumulation of Na^+ in the roots, while the content in the leaf remained low, even at the highest levels of salinity. In the case of K^+ it was vice versa as it accumulated more in the leaves. The higher Na^+ content in the roots also contributed to the maintenance of higher values of K^+/Na^+ ratio in the shoots, a very pertinent characteristic feature required for the salinity tolerance of glycophytes (Lacerda et al., 2003; Aquino et al., 2007). It was evident from the strong correlation observed between leaf K^+ content with [P_N] (r = 0.94) and biomass (r = 0.864) while strong negative correlation between leaf Na^+ with [P_N] (r=-0.947) and biomass (r=-0.913). It is paramount to point out that, although Na^+ can substitute for K^+ in the process of osmotic adjustment, it does not in specific functions of K^+ in plant metabolism (Hebbar et al., 2000). Thus, the maintenance of higher K^+/Na^+ ratios in the leaf tissues (Taiz & Zeiger, 2013), as observed in the present study at 10 % and 25 % SWS, reduces the impacts of salt

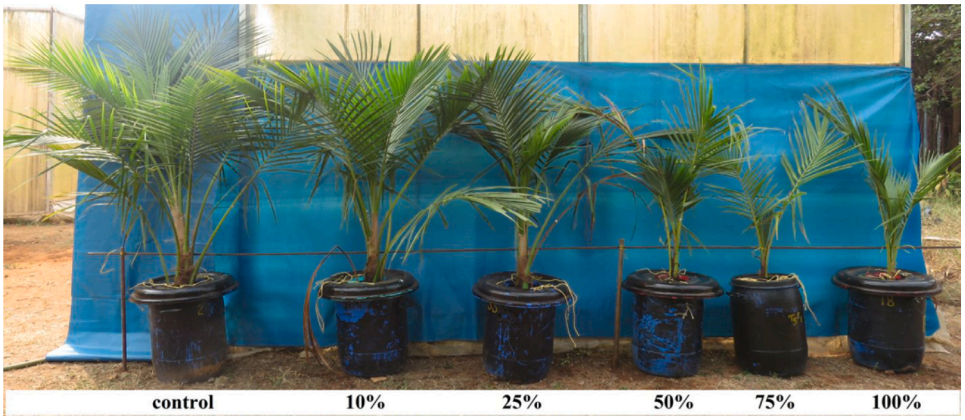

Fig. 2. Photograph showing the growth of thirteen month old hydroponically grown coconut seedlings. Three months after substitution of Hoagland solution by increasing substitution of sea water at 10, 25, 50, 75 and 100 % stunted the plant growth remarkably.

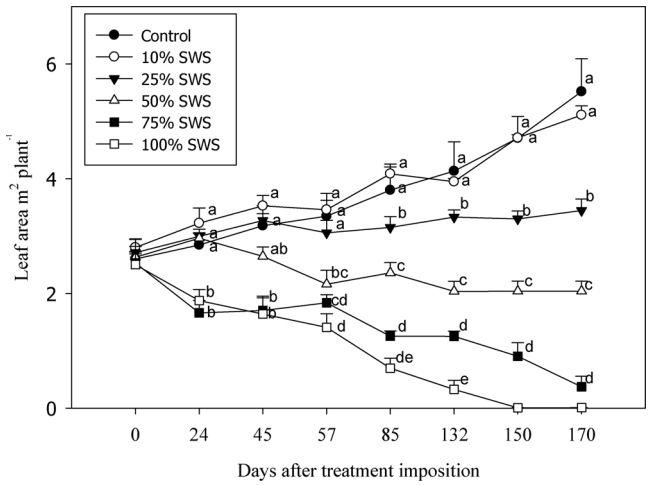

Fig. 3. Time course measurement of leaf area of ten month old hydroponically grown coconut seedlings treated with different sea water substitutions. Data are mean value of 3 replicates. At each observation data are analyzed using one way ANOVA. Mean with same letter represent non significance while different letter represent significance. Vertical lines represent standard error.

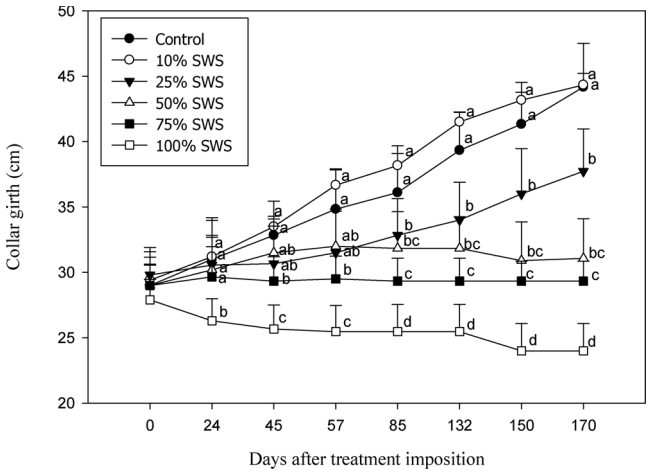

Fig. 4. Time course measurement of collar girth of ten month old hydroponically grown coconut seedlings treated with different sea water substitutions. Data are mean value of 3 replicates. At each observation data are analyzed using one way ANOVA. Mean with same letter represent non significance while different letter represent significance. Vertical lines represent standard error.

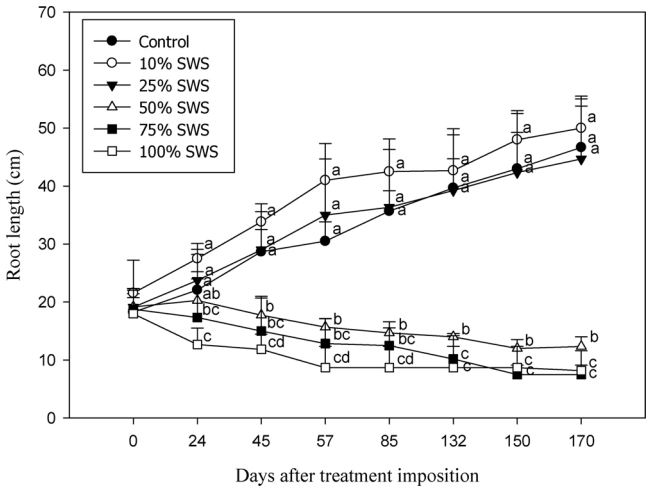

Fig. 5. Time course measurement of root length of ten month old hydroponically grown coconut seedlings treated with different sea water substitutions. Data are mean value of 3 replicates. At each observation data are analyzed using one way ANOVA. Mean with same letter represent non significance while different letter represent significance. Vertical lines represent standard error.

stress on the metabolism and production of photo assimilates (Aquino et al., 2007). As with leaf Na^+, the leaf Cl^- content also had strong negative correlation with [P_N] (r=-0.873) and biomass (r=-0.833). Therefore, in addition to the maintenance of the balanced K^+/Na^+ ratio in leaves, the level of tolerance to the increased chloride content in the leaves may also play an important role in salinity tolerance of coconut to the SWS (De Sedas et al., 2020).

In addition to ion homeostasis and compartmentalization, plants develop various mechanisms like biosynthesis of osmo-protectants and compatible solutes, activation of antioxidant enzyme and synthesis of antioxidant compounds etc., to survive under salinity stress. Leaf total sugar and reducing sugar as osmotic solutes did not increase significantly though accumulation of soluble carbohydrates is widely recognized as an important adaptive mechanism of plants subjected to salinity stress (Docimo et al., 2020; Murakezy et al., 2003; Parida et al., 2002; Parvaiz and Satyawati, 2008a,b; Rahneshan et al., 2018). Though, seedlings of coconut demonstrated significant accumulation of total polyphenol and antioxidant potential but it was only at 50 % SWS and beyond, at that concentration seedling growth was severely affected as a consequence there was negative association between polyphenol content and biomass (r=-0.803). In general, salinity tolerance is positively correlated with the activity of antioxidant enzymes, such as superoxide

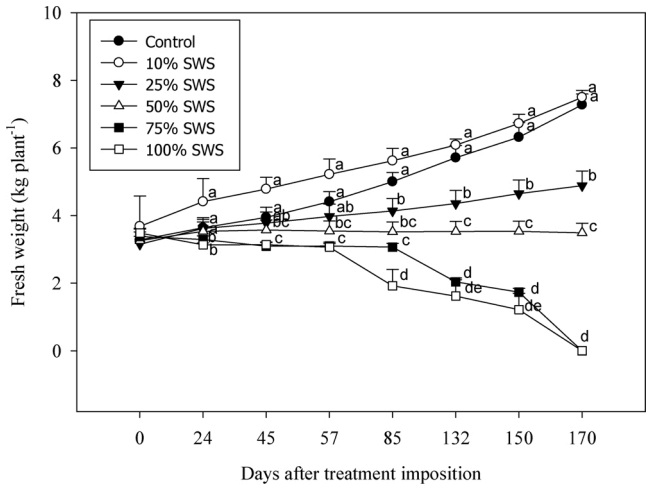

Fig. 6. Time course measurement of fresh weight of ten month old hydroponically grown coconut seedlings treated with different sea water substitutions. Data are mean value of 3 replicates. At each observation data are analyzed using one way ANOVA. Mean with same letter represent non significance while different letter represent significance. Vertical lines represent standard error.

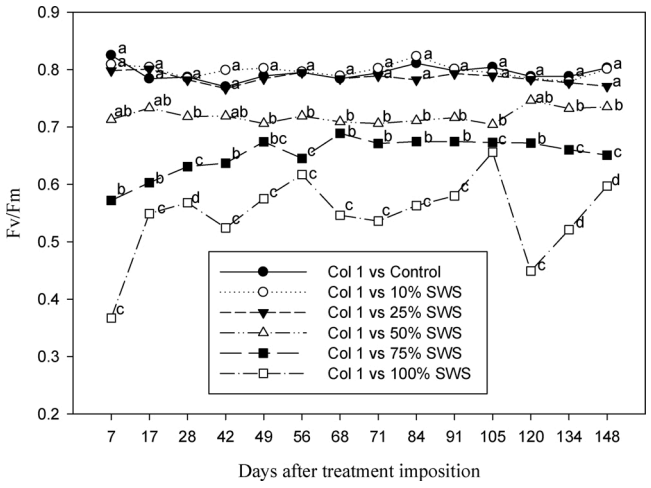

Fig. 8. Time course measurement of Fv/Fm of ten month old hydroponically grown coconut seedlings treated with different sea water substitutions. Data are mean value of 3 replicates. At each observation data are analyzed using one way ANOVA. Mean with same letter represent non significance while different letter represent significance.

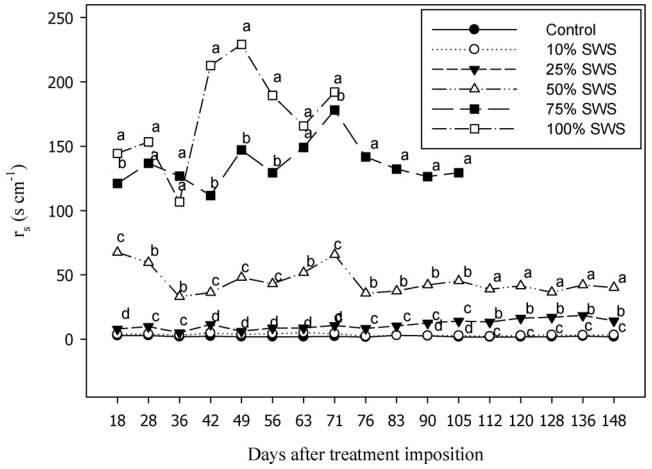

Fig. 7. Time course measurement of stomatal resistance of ten month old hydroponically grown coconut seedlings treated with different sea water substitutions. Data are mean value of 3 replicates. At each observation data are analyzed using one way ANOVA. Mean with same letter represent non significance while different letter represent significance.

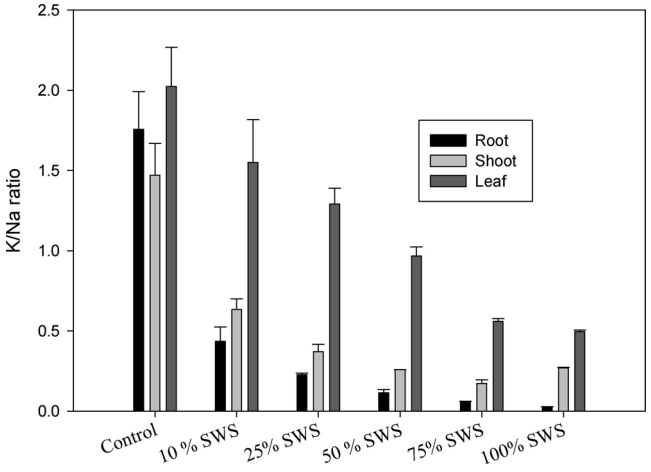

Fig. 9. K^+/Na^+ ratio of root, shoot and leaf of coconut seedlings with increasing level of sea water substitution. Vertical lines represent standard error.

dismutase (SOD), catalase (CAT), peroxidase (GPX), and ascorbate peroxidase (APX) and with the accumulation of non-enzymatic antioxidant compounds (Asada, 1999; Gupta et al., 2005). Similarly, the robust activities of antioxidant enzymes, accumulation of osmolytes, and oxidative markers in the roots and leaves of tetraploid volkamer lemon seedlings have been correlated with its salinity tolerance (Khalid et al., 2020). However, in our study biomass production under salinity had a strong negative correlation with SOD (r=-0.941), PPO (r=-0.934) implying antioxidant enzymes in coconut variety MGD had minimal protective role beyond 25 % SWS similar to the physiological behaviour and antioxidant responses of perennial halophyte *Crithmum maritimum* (Ben Amor et al., 2005).

To conclude, our study delineates the growth, physiological and biochemical responses of dwarf coconut seedlings subjected to sea water substitutions in a hydroponics system. This investigation reveals that coconut seedlings can tolerate sea water substitution to the extent of 8.32 dS m^{-1} EC of nutrient solution as no adverse effect on the physiology and biomass accumulation was observed. At 25 % SWS (16.32 EC) biomass production was decreased by 47 %. Both leaf area and P_N decreased but P_N is more sensitive to salinity. Exposing coconut seedlings to sea water substitutions of 50%–100% was found to be detrimental. Biomass fractionation during sea water substitution stress divulged that greater allocation of biomass to roots could be an adaptive strategy to tide over salt stress. Also, Na^+ uptake by the seedlings in sea water treatments was found to be accumulated more in root and shoot and very little is transported to leaves while maximum K^+ was transported to leaves resulting in the maintenance of balanced K/Na ratio in leaves which may be a salinity tolerance mechanism in coconut. However, from the findings it is clear that there is need to study the response at a narrow range between 0–25% SWS. There is also need to study the response in tall trees, which are more tolerant to abiotic stresses.

Funding

This study was funded by Indian Council of Agricultural Research (ICAR) (ICAR-CPCRI Project No: 1000766014).

CRediT authorship contribution statement

Hebbar K.B.: Conceptualization, Methodology, Formal analysis, Resources, Project administration, Supervision, Writing - original draft, Writing - review & editing. **Arya Santhosh:** Investigation, Validation, Data curation. **Abhin P. Sukumar:** Investigation, Validation, Data curation. **Neethu P.:** Investigation, Validation, Data curation. **Ramesh S.V.:** Investigation, Validation, Data curation, Writing - original draft, Writing - review & editing. **Selvamani V.:** Investigation, Validation, Data curation, Writing - original draft, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The authors gratefully acknowledge the funding for this study from Indian Council of Agricultural Research (ICAR) (ICAR-CPCRI Project No: 1000766014)

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