RESEARCH ARTICLE

Impact of sea-salt on morpho-physiological and biochemical responses in banana (*Musa acuminata* cv. Berangan)

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Abstract Banana is often grown in coastal-regions, and while known for its sensitivity towards seawater, little is documented on the effect of sea-salt on the growth, physiology and metal homeostasis. Here we report that banana plantlets exposed to sea-salt at extreme (average seawater concentration; 52.7 dS m⁻¹), severe (28.5 dS m⁻¹) or moderate (10.2 dS m⁻¹) salinity levels had reduced root length (2.0–6.0-fold), plant height (1.2–1.6-fold), leaf number (2.0–2.3-fold) and leaf area (3.3–4.0-fold) compared to control plantlets. Degradation of pigments (total chlorophyll: 1.3–12.3-fold, chlorophyll a: 1.3–9.2-fold; chlorophyll b: 1.3–6.9-fold lower and carotenoids: 1.4–3.7-fold lower) reflected vulnerability of photosystems to salt stress. Relative water content showed a maximum decrease of 1.5-fold in salt stress. MDA analysis showed sea-salt exposure triggers 2.3–3.5-fold higher lipid peroxidation. Metal content analysis showed a 73-fold higher Na value from roots exposed to extreme salinity compared to control plantlets. While phenotype was clearly affected, moderate salinity showed no significant alteration of macro (N, P, K and Ca) and micro (Fe, Mn and Cu) metal content. The antioxidant enzymes: SOD (3.2-fold), CAT (1.7-fold) and GR (6-fold) showed higher activity at moderate salinity level compared to control plantlets but lower activity at

severe (SOD: 1.3-fold; CAT: 1.5-fold; GR: 2-fold lower) and extreme seawater salinity (SOD: 1.5; CAT: 1.9; GR: 1.3-fold lower). Mild changes in growth and physiology at sea-salt levels equivalent to moderate seawater flooding, indicate that banana will survive such flooding, while extreme seawater inundation will be lethal. This data provides a reference for future salinity-mediated work in banana.

Keywords Antioxidant enzymes · Banana · Lipid peroxidation · Metal composition · Sea-salt · Seawater flooding

Introduction

Banana is a major staple food crop, just behind rice, wheat, and maize in world trade (Frison et al. 2004; Dash and Rai 2016; Perrier et al. 2011). Like other plants, due to a sessile nature, banana plants are likely to face multiple episodes of environmental stresses such as salinity and drought, during their lifespan. Banana is cultivated abundantly in coastal regions (Levillain et al. 2012; Vilardey et al. 2011), which are highly prone to rises in sea-level and resultant salinity problems (Murali and Kumar 2015). So far, 62 million hectares of the world's total 300 million hectares of irrigated land, has been salinized to some degree, and another 50 cm rise in sea-level is expected by 2100, which will swallow up 1.9 million more hectares (<http://www.cbc.ca/news/technology/saltwater-plants-1.3193535>). A steep decline in agricultural production, including banana, was recently reported in some coastal areas of South East Asian countries, due to saltwater intrusion, coupled with drought (FAO 23 August 2016). Hence, there is an urgent need to understand the response of banana to sea-salt stress, in

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order to optimise strategies to elevate the survival rate of banana in seawater affected areas.

The response of a plant species to a particular range of salinity depends on the morphological, physiological, and biochemical plasticity of the plant (Rangani et al. 2016). The survival fate of a plant species mainly depends on how effective the coordination is between physiological and metabolic pathways in order to mitigate the toxic effects of stress (Kumari et al. 2015). Tolerant species survive in salinity stress by sequestering salts in vacuoles and accumulating osmolytes in their cytoplasm, thus diluting the effect of ion toxicity while maintaining osmotic-balance (Hameed and Khan 2011; Hameed et al. 2015; Nedjimi 2014). Plants also express antioxidant enzymes, to scavenge reactive oxygen species (ROS) when exposed to increased levels of salinity (Bose et al. 2014). However, high salt stress can impair the capacity of these systems to detoxify ROS, triggering lethal damage (Hameed and Khan 2011). Although banana is known to be sensitive to salinity (El-Fadel et al. 2018; Israeli et al. 1986; Yano-Melo et al. 2003), little is known about the tolerance threshold. There is also a lack of data for the impacts on growth, photosynthesis, cell membrane integrity, metal accumulation and of the antioxidant system response in banana.

Increasing the electrical conductivity (EC) of irrigation water from 1 to 7 dS m⁻¹ in banana plantation has been reported to accelerate photosynthetic pigment degradation, which was followed by elevated necrosis at the lamina margins and yield loss in banana (*Musa* spp. AAA group cv. 'Grand Nain') (Shapira et al. 2009). Salinity studies on five varieties of banana: Pacovan, Nanicão, Caipira, FHIA18 and Calcuttá demonstrated differences in susceptibility levels to salt stress (Gomes et al. 2001). Two recent studies on salinity stress in banana performed in an in vitro condition (*Musa acuminata* cv. Berangan; Dikayani et al. 2017) and in a hydroponic system (Tap Maeo and Berlin genotypes; Willadino et al. 2017) indicated moderate susceptibility of this banana cultivar to salt stress. However, these studies were performed using only NaCl to produce salinity stress and so do not fully address the consequences of seawater intrusion (Van den Brand et al. 2015). Most salinity studies in plants use NaCl treatments, as this is the major component of sea-salt, while only a few studies e.g. of cowpea and maize (El-Shihaby et al. 2002), tomato (Hajer et al. 2006) and lettuce (Turhan et al. 2014) report results from experiments performed with sea-salt. The biological effects of NaCl and sea-salt have been shown to be dissimilar, as demonstrated in the aquatic macrophytes *Hydrilla verticillate*, *Najas indica* and *Najas gramenia* (Rout and shaw 2001). The superoxide dismutase (SOD) activity in aquatic macrophytes was higher in sea-salt-treated plants than NaCl-treated plants, while the activity of peroxidase was higher in NaCl-treated plants. Such

differences were explained as a consequence interaction of the Ca, Mg and K in sea-salt together with Na, as these metals not only influence water homeostasis but also act as co-factors for several vital enzymes and influence the expression of the antioxidative enzyme genes (Rout and Shaw 2001). Hence, to achieve more accurate data on the physiological effects of sea-water exposure, experiments with sea-salt can be used to explore a realistic effect of seawater inundation in banana.

Here, we investigated the impact of seawater salinity on plant height, root length, leaf number, leaf area, relative water content (RWC) and photosynthetic pigments of a popular Cavendish-type banana cultivar, *Musa acuminata* cv. Berangan (with triploid AAA genome) under conditions that simulate moderate (10.2 dS m⁻¹), severe (28.5 dS m⁻¹) and extreme (52.7 dS m⁻¹) levels of seawater exposure. The changes in the levels of the antioxidative enzymes, SOD, catalase (CAT) and glutathione reductase (GR), changes in lipid peroxidation and of metal content, were measured to obtain insight into the response of banana to sea-salt salinity.

Materials and methods

Plant material, salt treatment, and experimental setup

Banana (*Musa acuminata* cv. Berangan), (AAA) clonal plantlets were obtained from Felda Agricultural Services Sdn Bhd (Negeri Sembilan, Malaysia). One-month-old tissue culture-derived banana plantlets were acclimatized in black plastic bags (W × L × H; 10 cm × 10 cm × 25 cm) containing autoclaved soil and mushroom compost (3:1) for 10 days. Following that, plantlets of approximately equal height (between 15.4 and 15.9 cm tall) were selected and salinity stress treatments were carried out. Aquarium salt (Red Sea, China) (<https://www.redseafish.com/red-sea-salts/>), a mimic of seawater was used for salt treatment. Three different saline solutions were prepared for treatments; the extreme salinity treatment used salts at the equivalent of the average concentrations in seawater of 52.7 dS m⁻¹; the severe salinity treatment used salts at half the conductivity of seawater, 28.5 dS m⁻¹ and the moderately saline treatment was at 10.2 dS m⁻¹. Control plantlets (0 dS m⁻¹) were watered normally. For each treatment including the control, 20 replicates of plastic bags were prepared and all the experiments were repeated for five times (5 × 20 = 100 plantlets). Each plantlet was "watered" with 50 ml saline (treatment groups) or distilled water (control group) each day for 15 days. After 15 days, plantlets were watered with 50 ml of distilled water each day for a 10 days recovery period. The uprooted plantlets

were washed carefully in tap water to remove all soil debris from roots, prior to measurement of phenotypic parameters. The study was performed in the GP-BSL2 greenhouse (Plant Biotech Facility), University Malaya, Malaysia under natural light (12 h photoperiod) in a controlled temperature ($25 \pm 2^\circ\text{C}$) and relative humidity ($65 \pm 5\%$). The experiment was arranged in a randomized block design with twenty biological replicates in each treatment, and each treatment repeated five times.

Determination of growth parameters

Phenotypic parameters including plant height, root length, leaf number and leaf area were determined for sea salt-treated and control plantlets. For measurement of aerial and root biomass, root and aerial parts were excised from salt-treated and control plantlets, and the fresh weights were recorded immediately. The plant parts were wrapped in pre-weighed aluminium foil and dried in an oven at 75°C for 72 h before the dry weights were recorded. Total leaf area per plantlets was measured in both salt-treated and control plantlets using Digimizer software, version 5.0 (MedCalc Software, Belgium).

Quantification of leaf relative water content (RWC%)

The relative water content (RWC) of the leaves was measured according to the method suggested by Barrs and Weatherley (1962). The leaves were collected from salt-treated and control plantlets. The leaf fresh weight (LFW) was measured immediately after sampling, and then, the leaves were immersed in distilled water for 8 h at room temperature ($25 \pm 2^\circ\text{C}$). The leaves were then blotted to dry and the leaf turgid weight (LTW) was taken prior to drying in an oven at 75°C for 72 h. After the incubation period, the leaf dry weight (LDW) was noted. The leaf RWC was calculated using the following formula:

$$\text{RWC (\%)} = \frac{(\text{LFW} - \text{LDW})}{(\text{LTW} - \text{LDW})} \times 100$$

Determination of cell death

Evan's blue staining of the leaf was used to detect cell death according to the method suggested by Qin et al. (2017). Briefly, banana leaves were stained with 0.2% (W/V) Evans blue at 25°C for 8 h. The leaves were washed with water to remove excess stain and then bleached in warm methanol: acetic acid: glycerol (3:2:1) solution to remove the chlorophyll (Rangani et al. 2016). The leaves were blotted dry and scanned using a DocuCentre-IV 2060 scanner (Fuji Xerox, Japan).

Determination of lipid peroxidation

The extent of cell membrane damage was estimated by measuring the malondialdehyde (MDA) content which is a product of lipid peroxidation in plants under environmental stress conditions, following the method suggested by Draper and Hadley (1990). Leaves (100 mg) were homogenized in 2 ml of 0.1% (w/v) TCA solution. The homogenate was centrifuged at 13,000 rpm for 15 min at 4°C , the supernatant was collected, and 500 μl of the supernatant was mixed with 2 ml of 0.5% (w/v) TBA prepared in 20% (w/v) TCA. The mixture was incubated at 95°C for 30 min, and the reaction was stopped by placing the tubes on ice. Samples were centrifuged at 10,000 rpm for 5 min, and the absorbance of the supernatant was recorded at 532 nm. The concentration of MDA was calculated from the extinction coefficient of $155 \text{ mM}^{-1} \text{ cm}^{-1}$.

Determination of photosynthetic pigment

The effect of increasing salinity on photosynthetic activity was determined by measuring chlorophyll a (Chl a) and chlorophyll b (Chl b) content according to the method suggested by Inskeep and Bloom (1985) and carotenoid contents according to the method suggested by Chamovitz et al. (1993). Leaves (100 mg) from saline stress recovered plantlets were homogenized in 2 ml of pre-chilled 100% N,N-dimethylformamide (DMF) in the dark and the homogenate was centrifuged at 13,000 rpm for 10 min. The supernatant was collected and diluted five-fold and absorbance was measured at wavelength 664, 647, and 461 nm. The Chl a, Chl b and carotenoid content were estimated using the following formulae:

$$\text{Chl a} = 12.25A_{663.2} - 2.79A_{646.8} (\mu\text{g mL}^{-1} \text{ solution})$$

$$\text{Chl b} = 21.50A_{646.8} - 5.10A_{663.2} (\mu\text{g/mL}^{-1} \text{ solution})$$

$$\text{Total carotenoids} = \frac{1000A_{470} - 1.82Ca - 85.02Cb}{198}$$

(μg per mL solution)

where A = absorbance in 1.00 cm cuvettes and Chl = mg L^{-1} .

Determination of proline content

Proline concentration was determined according to the method described by Bates et al. (1973). Leaves (500 mg) were homogenized with 5 ml of sulfosalicylic acid (3%). The homogenate was filtered through Whatman No. 1 filter paper and the volume was raised to 10 ml with sulfosalicylic acid. Each aliquot of 2 ml of filtrate was incubated with 2 ml of glacial acetic acid and 2 ml of ninhydrin reagent and boiled in a water bath at 100°C for 30 min.

The reaction mixture was cooled to room temperature and 6 ml of toluene was added and the mixture vortexed. The absorbance of the mixture was measured at a wavelength of 570 nm.

Quantification of ROS and determination of ROS scavenging enzyme [superoxide dismutase (SOD), catalase (CAT) and glutathione reductase (GR)] activity

Hydrogen peroxide (H_2O_2) was quantified according to the method described by Sagisaka (1976). Leaves (200 mg) were homogenized in 5 ml of 5% trichloroacetic acid (TCA). The homogenate was centrifuged at 13,000 rpm for 10 min. Next, 40 ml of 50% TCA, 20 ml of potassium thiocyanate and 40 ml of ferrous ammonium sulphate were added to the supernatant to determine H_2O_2 levels. The absorbance was measured at 480 nm.

Superoxide ($\text{O}_2^{\cdot -}$) was estimated according to the method described by Muneer et al. (2014). Leaves (200 mg) were homogenized in phosphate buffer (pH 7.5) and centrifuged at 13,000 rpm for 5 min. Next, 1 ml of 10 mM hydroxylamine hydrochloride and phosphate buffer (pH 7.8) was added to homogenate and incubated at 25 °C for 1 h. After incubation, the mixture was added with 0.5 ml of 17 mM α -aminobenzene sulphonic acid and 0.5 ml of 7 mM α -naphthylamine solution. The reaction mixture was incubated for 20 min at 25 °C and absorbance measured at a wavelength of 530 nm.

SOD activity was quantified based on its ability to inhibit photochemical reduction of nitroblue tetrazolium (NBT) at 560 nm according to the method suggested by Gupta et al. (1993). Leaf tissues (100 mg) were homogenized in 1 ml of 50 mM phosphate buffer (pH 7.8). The homogenate was centrifuged at 13,000 rpm for 15 min at 4 °C and 100 μl supernatant was added into a 3 ml reaction mixture [50 mM phosphate buffer (pH 7.8), 13 μM methionine, 63 μM NBT and 1.3 μM riboflavin]. The reaction was started by keeping the solution under 30 W white fluorescent light and was stopped after 10 min by turning off the light and measured the absorbance at 560 nm.

CAT activity was determined based on the initial rate of reduction in H_2O_2 at 240 nm according to the method suggested by Aebi (1984). Leaf tissues (100 mg) were homogenized in 1 ml of 50 mM phosphate buffer (pH 7.8). The homogenate was centrifuged at 13,000 rpm for 15 min at 4 °C and 100 μl of supernatant was added into a 3 ml reaction mixture [3.2 mM H_2O_2 in 50 mM phosphate buffer (pH 7.0)] and the resultant mixture was used to measure the absorbance at 240 nm.

GR activity was quantified by the oxidation of NADPH at 340 nm according to the method described by Smith

et al. (1989). Leaf tissues (100 mg) were homogenized in 1 ml of 50 mM phosphate buffer (pH 7.6). The homogenate was centrifuged at 13,000 rpm for 15 min at 4 °C and 50 μl was added into a 1 ml reaction mixture (1 mM oxidized glutathione and 2 mM NADPH) and was used to measure the absorbance at 340 nm.

Analysis of physico-chemical parameters of saline water, soil and root samples

The electronic conductivity of prepared sea-salt solutions was measured using a conductivity meter (Oakton, Thermofisher Scientific, USA) and pH was measured using a pH meter (Satorius, Germany). After harvesting of plantlets, the rhizospheric soil was collected and analysed for pH, organic matter, total N, available P and K by following procedures of Piper (1950) and Bremner (1965) at Sime Darby Seeds & Agriculture Services Sdn. Bhd, Malaysia.

For metal analysis, root samples were dried in an oven at 75 °C for 48 h. After drying, 50 mg of samples were homogenized and then 30 mg of ground root material was placed in a 50 mL metal free tube. Then, the samples were digested at 100 °C with 10 mL of nitric acid for 9 h and the final volume of 50 mL was made up with Milli-Q water. Then, 5 mL of the sample was aliquoted in a metal free tube and was centrifuged at 10,000 rpm for 2 min in order to obtain a homogeneous dispersion. Next, 200 μL sample was aliquoted in another tube and diluted to 2 mL (10 $\times$ dilution) using 5% hydrogen peroxide and 1% nitric acid solution. An internal standard (indium, 20 ppb) was also added to the solution. Aliquots of this solution were used for the determination of metal concentrations. Metals were measured using a Perkin-Elmer NexIon 350X ICP-MS operating in a dual detector mode. Blank subtraction was applied after internal standard correction. A three point-calibration curve was used to quantify the compounds. The accuracy of the ICP-MS analytical protocol was periodically evaluated via the analysis of certified reference materials (serum & urine toxicology controls) from UTAK laboratories, Inc. and water sample from NIST.

Statistical analysis

All statistical analyses were performed using SPSS Version 24.0 for Windows (SPSS Inc., Chicago, IL, USA). Multiple comparisons were conducted with Tukey HSD test ($p \leq 0.05$ was considered as significant).

Results

Seawater salinity impairs growth and biomass accumulation in banana

The pH of the salt solutions used for treatment ranged from neutral (6.99) in control (distilled water) to alkaline (9.28–9.33) for the sea-salt solutions (Table 1). Analysis of soil samples collected after harvesting of treated banana plantlets showed near neutral pH (6.9–7.0), low EC and low salt content compared to the sea-salt solutions used in the treatment (Table 2). The effects of sea-salt on plant growth were determined by measuring plant height, root length, leaf number, leaf area, shoot and root biomass (fresh and dry weight). Higher concentrations of sea-salt resulted in increasing symptoms of stress in banana plantlets (Fig. 1) and reduced plant growth (Fig. 2). Plant height reduced with an increase in salinity level (Fig. 2a) and was 1.2-fold lower in plantlets exposed to moderate (10.2 dS m^{-1}) salinity compared to control plantlets and 1.6-fold lower for the plantlets exposed to extreme (52.7 dS m^{-1}) salinity as compared to control plantlets (Fig. 2a). Root growth reduced with increasing salinity level (Figs. 2b, 3) with 2-fold lower root length exposed to moderate salinity and 4.6-fold lower at extreme (52.7 dS m^{-1}) salinity as compared to control plantlets (Figs. 2b, 3). The elevated salt level was also observed to induce necrosis in older leaves, followed by rapid senescence, leaf drop and suppression of new leaf emergence, which was reflected as low leaf number and much-reduced leaf areas (Fig. 2d). Salt stress injury was visible in leaf lamina with the spreading of yellow chlorosis from the edge of necrotic zones and subsequently dry and shrivelled leaves, visible in Evan blue staining (Fig. 4). Aerial and root biomass, determined by fresh and dry weight, also reflected a detrimental effect of sea-salt on banana plantlet growth (Table 3). The lowest dry biomass accumulation was observed in plantlets exposed to extreme salinity, where, aerial dry biomass accumulation was 4.6-fold lower and root dry biomass accumulation recorded was 6-fold lower compared to control plantlets (Table 3).

Seawater salinity reduces photosynthetic pigment content in leaves of banana

Photosynthetic pigment content is commonly used to evaluate the extent of damage to a photosynthetic system under various environmental stress conditions (Falqueto et al. 2017; Zushi and Matsuzoe 2017). Quantification of photosynthetic pigments (chlorophyll and carotenoid) from banana leaves under various sea-salt treatments showed significantly lower values for total chlorophyll content (Fig. 5a), chlorophyll a (Fig. 5b), chlorophyll b (Fig. 5c) and carotenoid content (Fig. 5d) with an increase in salinity level. The total chlorophyll content showed a maximum decrease (12.3-fold lower) in extreme salinity treated banana plantlets compared to control plantlets. Similarly, chlorophyll a was found to be lower by 9.2-fold (Fig. 5b) and chlorophyll b was 6.9-fold lower in extreme salinity treated compared to control plantlets (Fig. 5c).

Seawater salinity induces imbalance of banana leaf water status, proline content and accelerates oxidative degradation of lipids

The RWC provides a measurement of the water status of the plant. The analysis of RWC showed that an increase in salinity induces a reduction in RWC with the largest decrease in RWC (1.5-fold lower than the control) observed in plantlets exposed to 52.7 dS m^{-1} sea-salt salinity (Fig. 6a). In plants, stresses that perturb osmotic balance trigger an accumulation of organic osmolytes such as proline, to maintain cellular homeostasis. Proline content was significantly higher in banana plantlets exposed to moderate 10.2 dS m^{-1} (1.5-fold), severe 28.5 dS m^{-1} (1.9-fold) and extreme 52.7 dS m^{-1} (2.2-fold) salinity compared to control plantlets (Fig. 6c). Stress triggers lipid peroxidation via excessive production of ROS (Taïbi et al. 2016). Lipid peroxidation is commonly estimated by measuring MDA (Gawel et al. 2004). The MDA level showed a significant rise with an increase in salinity. MDA levels increased significantly in plantlets exposed to severe 28.5 dS m^{-1} (2.3-fold) and extreme 52.7 dS m^{-1} (3.5-fold) salinity compared to control plantlets (Fig. 6b).

Table 1 Element level, electronic conductivity and pH of sea-salt solution used for banana treatment

Electronic conductivity (dS m^{-1})	Concentration of NaCl in sea salt solution (parts per thousand ^a)	Ca (ppm)	Mg (ppm)	K (ppm)	pH
0.001	0	0	0	0	6.99
10.2	5.84	64–67	190–200	57–61	9.28
28.5	17.5	180–190	538–568	163–173	9.30
52.7	33.3	313–330	935–987	283–300	9.33

^aParts per thousand (ppt) is equivalent to “g/L” concentration of NaCl

Table 2 Physical and chemical properties of the rhizosphere soil irrigated with various concentrations of sea-salt solution collected after harvesting of plants

Salinity of treated solution (dS m ⁻¹)	pH	Electronic conductivity (dS m ⁻¹)	Total N (%)	Exchangeable Na (me%)
0	6.9	1.05	0.077	0.20
10.2	6.9	1.65	0.071	3.00
28.5	6.8	3.29	0.061	7.65
52.7	7.0	3.44	0.056	8.91

Fig. 1 Morphological changes in banana plantlets following exposure to 10.2 dS m⁻¹, 28.5 dS m⁻¹ and 52.7 dS m⁻¹ salinity for a period of 15 days followed by a recovery period of 10 days

Moderate levels of seawater salinity induce banana antioxidant enzyme activity

Elevated salinity results in osmotic stress in plant cells, which accelerates the accumulation of ROS such as H₂O₂ and O₂⁻¹ (Gill and Tuteja 2010) (Pang and Wang 2008). Analysis of H₂O₂ levels in banana plantlets showed significant higher levels with increases in salinity. H₂O₂ levels increased in plantlets exposed to moderate 10.2 dS m⁻¹ (2.1-fold), severe 28.5 dS m⁻¹ (6.8-fold) and extreme 52.7 dS m⁻¹ (9.9-fold) salinity compared to control plantlets (Fig. 7a). O₂⁻¹ was also significantly higher in plantlets exposed to moderate 10.2 dS m⁻¹ (3.4-fold), severe 28.5 dS m⁻¹ (7.3-fold) and extreme 52.7 dS m⁻¹ (10.1-fold) salinity compared to control plantlets (Fig. 7b).

As antioxidant enzymes act as a prime line of defence against ROS in plant cells, stress tolerance of a species is associated with the efficient production of antioxidant enzymes to scavenge ROS (reviewed in Das and Roychoudhury 2014). The antioxidant enzymes; SOD, CAT

and GR measured in banana leaves, showed highest activity in plantlets exposed to moderate levels of sea-salt and no significant difference in enzyme activities was observed in plantlets exposed to severe or extreme levels of sea-salt (Fig. 8a–c). The activity of SOD in banana was observed to be 3.2-fold higher (Fig. 8a), CAT activity was observed to be increased by 1.7-fold and GR activity by 6-fold in plantlets exposed to moderate salinity compared to control plantlets.

Seawater salinity alters the metal composition of banana roots

The Na content in the root of sea-salt-treated banana plantlets was found to successively increase with increasing salinity level (Table 4). The Na content was observed to be 8.1-fold higher in plant roots exposed to moderate salinity, 35.1-fold higher in severe salinity, and 73-fold higher in extreme salinity compared to the roots of control plantlets (Table 4). Other metals that also increased in the

◀**Fig. 2** Changes in **a** plant height; **b** root length; **c** no. of leaves and **d** leaf area of one month old banana plantlets following exposure to 10.2 dS m⁻¹, 28.5 dS m⁻¹ and 52.7 dS m⁻¹ salinity for a period of 15 days, followed by a recovery period of 10 days. The different letters on the top of the error bars show statistically different means at $p \leq 0.05$

Fig. 3 Morphological changes in banana root following exposure to 10.2 dS m⁻¹, 28.5 dS m⁻¹ and 52.7 dS m⁻¹ salinity for a period of 15 days, followed by a recovery period of 10 days

roots as sea-salt level concentrations increased were Mn and Pb (Table 4). In contrast, the metals K, Ca, Mg and Co decreased in response to increasing sea-salt levels, while there were no significant differences for Fe, Cu or P. The K, Ca, Mg, Fe, Mn, Cu, P and Co content was observed to be not significantly different in roots of plantlets exposed to moderate salinity compared to those of control plantlets, but significant changes were observed in plantlets exposed to severe salinity (K: 1.2-fold and Ca: 1.5-fold lower Mg: 2-fold, and Co 2-fold lower compared to control) and at extreme 52.7 dS m⁻¹ salinity (K: 1.5-fold; Ca: 3.1-fold, Mg: 4.7-fold, and Co: 2.5-fold lower compared to control) (Table 4).

Fig. 4 Evan blue staining in the leaves of banana plantlets following exposure to 10.2 dS m⁻¹, 28.5 dS m⁻¹ and 52.7 dS m⁻¹ salinity for a period of 15 days, followed by a recovery period of 10 days

Discussion

Banana is one of the most highly nutritious and easily grown low-cost fruits (Robinson and Saúco 2010) and is abundantly grown in coastal regions (Levillain et al. 2012; Vilardy et al. 2011). Recent climate change, increases in irrigation practices and seawater intrusion in coastal regions, contribute to increased salinization of soil, negatively impacting banana yields (Nair 2000) and contributing to human malnourishment (FAO 2017). Improvements in the economics and sustainability of banana farming can be supported with cultivars which can grow in salinized soil without much effect to yield, and these will be much needed in the face of climate change. As a first step to understand the responses of banana, we have systematically analysed the effect of sea-salt on morphology, photosynthetic pigments, cell membrane integrity, metal

composition and antioxidant enzyme activity of a popular commercial Cavendish-type banana cultivar, Berangan. As an improvement to models using NaCl for salinity studies, we simulated seawater intrusion using a commercial sea-salt at three levels of salinity: 52.7 dS m⁻¹ (effectively pure seawater) to mimic complete inundation with seawater, 28.5 dS m⁻¹ (roughly half-strength seawater) to mimic severe seawater flooding and 10.2 dS m⁻¹ to mimic moderate seawater flooding. The commercial sea-salt used for our study contains Na, Mg, Ca and K salts (<https://www.redseafish.com/red-sea-salts/>) (Table 1), designed to make solutions that are highly similar to seawater.

Treatment with the seawater flooding mimics resulted in significant morphological differences in banana, with stunted plant height (Figs. 1, 2a) and reduced root length (Figs. 2b, 3). The reduction in root length was more drastic (up to 4.6-fold lower compared to control plantlets) (Fig. 2b) than growth retardation in the aerial parts of the plantlets (up to 1.6-fold lower in plant height compared to control plantlets) (Figs. 1, 2a). Measurement of fresh and dry weight showed low biomass accumulation in response to sea-salt (Table 3). Low biomass accumulation has been associated with a reduction in the rate of photosynthesis in plants (reviewed in Kumar et al. 2018), which is consistent with our data, where the sea-salt-treated banana plantlets had reduced levels of photosynthetic pigments, with up to 12-fold lower chlorophyll content compared to control plantlets (Fig. 5a–c). Lower levels of photosynthetic pigments under environmental stress, indicate a stress-induced reduction in synthesis and/or rapid breakdown of the pigments in cells due to the formation of ROS (Ashraf 2003). This was clearly evident in banana plantlets exposed to increasing levels of seawater salinity, where photosynthetic pigments decreased (Fig. 5), while ROS levels increased (Fig. 7).

Among photosynthetic pigments analysed in the leaves of sea-salt-treated banana plantlets, carotenoid content was observed to be least affected by moderate and severe levels of salinity (Fig. 5d). The relatively lower degradation of carotenoid might be due to less reduction in biosynthesis and/or to a lower rate of degradation of the pigment and may provide some level of protection to banana plantlets

Table 3 Effect of sea salt on aerial and root fresh and dry weight of banana

Plant growth parameters	Salinity of treated solution (dS m ⁻¹)			
	0	10.2	28.5	52.7
Aerial fresh weight	7.47 ± 0.73 ^a	5.64 ± 1.11 ^b	3.98 ± 0.74 ^c	2.53 ± 1.26 ^d
Aerial dry weight	3.75 ± 0.57 ^a	2.01 ± 0.40 ^b	1.45 ± 0.32 ^c	0.82 ± 0.30 ^d
Root fresh weight	5.09 ± 0.55 ^a	3.22 ± 0.42 ^b	2.09 ± 0.33 ^c	1.61 ± 0.89 ^d
Root dry weight	0.96 ± 0.11 ^a	0.79 ± 0.05 ^b	0.45 ± 0.44 ^c	0.16 ± 0.04 ^d

Mean ± SD (n = 20) in the same row followed by the same letters are not significantly different ($p < 0.05$) by post hoc parametric test of Tukey HSD, $p < 0.05$

Fig. 5 Changes in photosynthetic pigments in the leaves of banana plantlets following exposure to 10.2 dS m⁻¹, 28.5 dS m⁻¹ and 52.7 dS m⁻¹ salinity for a period of 15 days, followed by a recovery period of 10 days. **a** Total chlorophyll; **b** chlorophyll a; **c** chlorophyll

b; **d** carotenoid. The values are mean $\pm$ SD ($n = 9$). The different letters on the top of the error bars show statistically different means at $p \leq 0.05$

exposed to seawater. A lower level of degradation of carotenoids, compared to that of chlorophylls was also reported in two pistachio cultivars, where it was suggested as a strategy of the plantlets to protect and stabilize photochemical processes by quenching ROS under stress conditions (Rahneshan et al. 2018). While salinity stress injury to banana plantlets from sea-salt treatment was observed to be less severe in aerial parts compared to roots, there was still a significant reduction in leaf area (3.3–4.9-fold) (Fig. 2d) and leaf number (1.3–3.8-fold) (Fig. 2c), becoming more pronounced with the increasing concentration of sea-salt. Reduction in leaf area on exposure to salinity has been also recorded in wheat (Howladar and Dennett 2015) and sugar cane (Simões et al. 2016). As increasing salt levels within the plant affect the water availability, reducing leaf area in response to salinity likely reflects a protective strategy by the banana plants to reduce the water lost via transpiration, as has been reported as a mechanism in turfgrass (McElroy and Kopsell 2009) and in the extreme halophyte, *Salvadora persica* (Rangani et al. 2016). At the cellular level, plants respond to water stress and perturbed osmotic balance through the accumulation of

osmolytes such as proline, reflecting stress-induced metabolic adaptation (Munns and Tester 2008). This has been reported for several plant species such as wheat (Poustini et al. 2007), tomato (Gharsallah et al. 2016) and Jerusalem artichoke (Huang et al. 2013) and was also observed in the leaves of the salinity exposed banana plantlets in our study (Fig. 6c). Exposure of banana plantlets to a moderate salinity level (10.2 dS m⁻¹) did not significantly alter levels of essential macro (N, P, K, Ca, and Mg) and micro (Fe, Cu, Mn and Co) metals in the roots (Table 4) in this experimental group and these plantlets also showed the mildest difference in morphology, compared to plantlets exposed to higher concentrations of sea salt. The plantlets in this group also showed a strong response of elevated levels of antioxidant enzymes (Fig. 8) consistent with the role of metal homeostasis in enzymatic function. Interestingly, the plantlets exposed to moderate (10.2 dS m⁻¹) and severe (28.5 dS m⁻¹) salinity level did not show significant difference in plant height, root length, leaf area, relative water content and lipid peroxidation. However, the biomass was found to be significantly lower in plants exposed at the higher of the two concentrations (Table 3) along with

Fig. 6 Changes in **a** relative water content (RWC), **b** lipid peroxidation level measured in terms of MDA content, **c** proline content in the leaves of banana plantlets following exposure to 10.2 dS m⁻¹, 28.5 dS m⁻¹ and 52.7 dS m⁻¹ salinity for a period of 15 days, followed by a recovery period of 10 days. The different letters on the top of the error bars show statistically different means at $p \leq 0.05$

a significant reduction in photosynthetic pigments (Fig. 5a–c) and increased levels of ROS (Fig. 7). Significantly, higher ROS formation in severe sea-salt exposed plantlets compared to moderate sea-salt exposed plantlets could explain the sudden reduction in antioxidant enzyme activity. So, while plantlets exposed to severe sea salt levels maintained their height, they were clearly not growing as vigorously, as reflected in biomass. This is

Fig. 7 Quantification of ROS content in the leaves of banana plantlets following exposure to 10.2 dS m⁻¹, 28.5 dS m⁻¹ and 52.7 dS m⁻¹ salinity for a period of 15 days, followed by a recovery period of 10 days. **a** Hydrogen peroxide, **b** superoxide. The different letters on the top of the error bars show statistically different means at $p \leq 0.05$

similar to *Ziziphus spina-christi* (Sohail et al. 2009) where, the threshold level for response as measured by one biological characteristic (such as height) is different to that for others (such as biomass). The plantlets exposed to extreme salinity level showed significant changes in plant phenotype, biomass, relative water content and lipid peroxidation. The significant reduction in the levels of essential metals, had apparently impaired antioxidant enzyme activity, which would explain the more severe morphological changes in this groups of plantlets.

Together, these results indicate that exposure to low levels of seawater, as might be encountered in flooding resulting from high tides accompanied by heavy rain, can be withstood by this cultivar of banana, albeit with much reduced growth. While essential metals are crucial for normal functioning, overall growth, photosynthesis and respiration in plants (reviewed in Grotz and Guerinet 2006; reviewed in Tripathi et al. 2014), a concentration outside of a plant's sufficiency range leads to overall growth retardation and even lethal damage due to either a deficiency or

Fig. 8 Salinity persuaded changes in the activity of antioxidative enzymes in the leaves of banana plantlets following exposure to 10.2 dS m⁻¹, 28.5 dS m⁻¹ and 52.7 dS m⁻¹ salinity for a period of 15 days followed by a recovery period of 10 days. **a** superoxide dismutase (SOD); **b** catalase (CAT); **c** glutathione reductase (GR). The different letters on the top of the error bars show statistically different means at $p \leq 0.05$

toxicity (Küpper and Andresen 2016). Thus, the relatively small differences in the metal content of the plantlets exposed to the lowest concentration of sea-salt, still resulted in quite large differences in the phenotype, in terms of leaf area and root length, over time. The response of banana in our study supports this as an “indicator” plant, characterised by high rates of Na accumulation (up to 73-fold higher) in roots with increases in salinity level (Table 4). Thus, banana is distinct from “excluder” type plants, which either actively remove excess metals to maintain a constant concentration in the plant cells

(halophytes) or “hyperaccumulator” type plants which actively accumulate metals in their shoots without showing significant signs of toxicity (Küpper and Andresen 2016).

Maintaining metal homeostasis is strenuous for plants under saline stress, as the accumulation of toxic Na interferes with the plant’s ability to control absorption of other ions (Negrão et al., 2017). The relatively low levels of K, Ca and Mg metals in banana roots after exposure to severe and extreme salinity levels (Table 4) are similar to observations reported for *Lotus creticus* (Rejili et al., 2007) and *Broussonetia papyrifera* (Zhang et al. 2013), where increases in accumulation of Na correlated with reduced absorption of K, Ca and Mg. The higher level of toxic heavy metal Pb in banana root are similar to the studies reported for River Red gum tree (*Eucalyptus camaldulensis* Dehnh.) (Nawaz et al. 2016) and ornamental kale (*Brassica oleracea*) (Haghighi et al. 2016), where increased mobility of Pb in saline condition was correlated with the replacement of Ca by Na, as Ca competes with Pb for sorption sites (Lutts and Lefèvre 2015). Reduced absorption of other essential metals has been explained as a consequence of substitution of Na for K (reviewed in Maathuis 2013), or Ca (reviewed in White and Broadley 2003) or Mg (reviewed in Guo et al. 2016) in the redox centre of enzymes. Such replacement of essential cofactor metals in enzymes of plants exposed to toxic levels of Na, leads to changes in associated metabolic pathways (Gill and Tuteja 2010), which hinder normal cellular functions and growth processes, induce oxidative damage (Tewari et al. 2004) and weaken the cell membrane integrity of plants (reviewed in Das and Roychoudhury 2014; Taïbi et al. 2016).

Symptoms of this type of damage were evident in banana exposed to severe and extreme levels of sea-salt, with the weak activity of the antioxidant enzymes SOD, CAT and GR (Fig. 8a–c) and high lipid peroxidation levels (Fig. 6b) compared to plantlets exposed to moderate levels of sea-salt. A decrease in activity of SOD, CAT and GR together with increased lipid peroxidation was also reported for *Broussonetia papyrifera* (Zhang et al. 2013) and *Nerium oleander* at levels of salinity beyond their tolerance levels (Kumar et al. 2017).

Conclusion

Based on morphological, physiological, and biochemical responses, the banana *Musa acuminata* cv. Berangan is unable to tolerate sea-salt at the levels likely to be experienced during moderate to severe flooding with seawater. Following exposure to moderate (10.2 dS m⁻¹) levels of sea-salt, banana plantlets showed the least severe changes in morphological (plant height, root length, number of leaves), physiological (photosynthetic pigments and

Table 4 Impact of sea-salt on metal composition of banana roots

Salinity of treated solution (dS m ⁻¹)	Na (mg g ⁻¹ DW)	K (mg g ⁻¹ DW)	Ca (mg g ⁻¹ DW)	Mg (mg g ⁻¹ DW)	Fe (mg g ⁻¹ DW)	Mn (mg g ⁻¹ DW)	Cu (ug g ⁻¹ DW)	P (mg g ⁻¹ DW)	Co (ug g ⁻¹ DW)	Pb ug g ⁻¹ DW)
0	0.37 ± 0.06 ^a	61.97 ± 1.12 ^a	9.76 ± 0.41 ^a	5.99 ± 0.58 ^a	1.72 ± 0.01 ^a	0.39 ± 0.13 ^a	4.10 ± 2.84 ^a	2.71 ± 0.18 ^a	0.20 ± 0.10 ^a	0.61 ± 0.08 ^a
10.2	3.00 ± 1.00 ^b	59.74 ± 2.82 ^a	8.16 ± 0.26 ^a	5.39 ± 0.88 ^a	1.64 ± 0.28 ^a	0.32 ± 0.05 ^a	4.09 ± 1.00 ^a	1.99 ± 0.59 ^a	0.19 ± 0.08 ^a	0.81 ± 0.05 ^b
28.5	13.00 ± 4.00 ^c	50.23 ± 3.42 ^b	6.64 ± 1.87 ^b	2.99 ± 2.00 ^b	1.55 ± 0.53 ^a	0.45 ± 0.03 ^a	4.04 ± 1.03 ^a	2.70 ± 0.96 ^a	0.10 ± 0.06 ^b	1.07 ± 0.03 ^c
52.7	27.00 ± 1.00 ^c	41.00 ± 3.87 ^c	3.12 ± 0.26 ^c	1.27 ± 0.51 ^b	0.92 ± 0.01 ^a	0.83 ± 0.16 ^b	4.04 ± 0.45 ^a	1.63 ± 0.38 ^a	0.08 ± 0.04 ^b	1.28 ± 0.09 ^d

Mean ± SD (n = 20) in the same row followed by the same letters are not significantly different ($p < 0.05$) by post hoc parametric test of Tukey HSD, $p < 0.05$

relative water content) and biochemical (MDA, SOD, CAT, GR and metals) parameters, ensuring plant adaptation and survival, however, the plants were short with much reduced leaf area and root length. Banana plantlets exposed to severe (28.5 dS m⁻¹) and extreme (52.7 dS m⁻¹) levels of sea-salt showed lethal damage, reflected by ROS accumulation, increase in osmolyte, loss of cell membrane integrity, cell death and by impaired photosynthetic pigment levels, metal homeostasis and antioxidant systems. This study provides specific data reflecting the morphophysiological and biochemical responses underlying the sensitivity of banana plants to sea-salt stress and will serve as the foundation for salinity tolerance research in banana.

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References

- Aebi H (1984) Catalase in vitro. *Methods Enzymol* 105:121–126. [https://doi.org/10.1016/S0076-6879\(84\)05016-3](https://doi.org/10.1016/S0076-6879(84)05016-3)
- Ashraf M (2003) Relationships between leaf gas exchange characteristics and growth of differently adapted populations of blue panic grass (*Panicum antidotale* Retz.) under salinity or waterlogging. *Plant Sci* 165:69–75. [https://doi.org/10.1016/S0168-9452\(03\)00128-6](https://doi.org/10.1016/S0168-9452(03)00128-6)
- Barrs HD, Weatherley PE (1962) A re-examination of the relative turgidity technique for estimating water deficits in leaves. *Aust J Biol Sci* 15:413–428. <https://doi.org/10.1071/B19620413>
- Bates LS, Waldren RP, Teare ID (1973) Rapid determination of free proline for water-stress studies. *Plant Soil* 39:205–207
- Bose J, Rodrigo-Moreno A, Shabala S (2014) ROS homeostasis in halophytes in the context of salinity stress tolerance. *J Exp Bot* 65(5):1241–1257. <https://doi.org/10.1093/jxb/ert430>
- Bremner JM (1965) Organic forms of nitrogen. In: Black CA et al (eds) *Methods of soil analysis*. Part II. American Society of Agronomy, Madison, Wisconsin, USA, pp 1238–1255
- Chamovitz D, Sandmann G, Hirschberg J (1993) Molecular and biochemical characterization of herbicide-resistant mutants of cyanobacteria reveals that phytoene desaturation is a rate-limiting step in carotenoid biosynthesis. *J Biol Chem* 268:17348–17353
- Das K, Roychoudhury A (2014) Reactive oxygen species (ROS) and response of antioxidants as ROS-scavengers during environmental stress in plants. *Front Environ Sci* 2:53. <https://doi.org/10.3389/fenvs.2014.00053>
- Dash PK, Rai R (2016) Translating the banana genome to delineate stress resistance, dwarfing, parthenocarp and mechanisms of fruit ripening. *Front Plant Sci* 7:1543. <https://doi.org/10.3389/fpls.2016.M01543>
- Dikayani A, Nuraini A, Qosim WA (2017) Response of shoot and root in vitro cultures of banana plant (*Musa acuminata* L.) cv

- Barangan to salinity stresses. *Asian J Agric Res* 11:103–107. <https://doi.org/10.3923/ajar.2017.103.107>
- Draper HH, Hadley M (1990) Malondialdehyde determination as index of lipid peroxidation. *Methods Enzymol* 186:421–431
- El-Fadel MU, Deeb T, Alameddine I, Zurayk R, Chaaban J (2018) Impact of groundwater salinity on agricultural productivity with climate change implications. *Int J Sustain Dev Plan* 13:445–456. <https://doi.org/10.2495/SDP-V13-N3-445-456>
- El-Shihaby OA, Younis ME, El-Bastawisy ZM, Nemat Alla MM (2002) Effect of kinetin on photosynthetic activity and carbohydrate content in waterlogged or seawater-treated *Vigna sinensis* and *Zea mays* plants. *Plant Biosyst* 136:277–290
- Falqueto AR, da Silva Júnior RA, Gomes MT, Martins JP, Silva DM, Partelli FL (2017) Effects of drought stress on chlorophyll a fluorescence in two rubber tree clones. *Sci Hortic* 224:238–243. <https://doi.org/10.1016/j.scienta.2017.06.019>
- FAO (2007) Advances in the assessment and monitoring of salinization and status of biosaline agriculture. Dubai, United Arab Emirates 26–29 November. Accessed at <http://www.fao.org/3/i1220e/i1220e.pdf>
- Frison EA, Escalant JV, Sharrock S, Jain SM, Swennen R (2004) The global Musa genomic consortium: a boost for banana improvement. *Banana improvement: cellular, molecular biology, and induced mutations*, pp 341–349
- Gawel S, Wardas M, Niedworok E, Wardas P (2004) Malondialdehyde (MDA) as a lipid peroxidation marker. *Wiadomosci lekarskie* (Warsaw, Poland: 1960) 57:453–455
- Gharsallah C, Fakhfakh H, Grubb D, Gorsane F (2016) Effect of salt stress on ion concentration, proline content, antioxidant enzyme activities and gene expression in tomato cultivars. *AoB Plants* 1:8. <https://doi.org/10.1093/aobpla/plw055>
- Gill SS, Tuteja N (2010) Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. *Plant Physiol Biochem* 48:909–930. <https://doi.org/10.1016/j.plaphy.2010.08.016>
- Gomes EWF, Willadino L, Martins LSS, Camara TR (2001) The effects of salinity on five banana genotypes (*Musa* spp). In: Horst WJ et al (eds) *Plant nutrition. Developments in plant and soil sciences*, vol 92. Springer, Dordrecht, pp 410–411
- Grotz N, Guerinot ML (2006) Molecular aspects of Cu, Fe and Zn homeostasis in plants. *BBA-Mol Cell Res* 1763:595–608. <https://doi.org/10.1016/j.bbamcr.2006.05.014>
- Guo W, Nazim H, Liang Z, Yang D (2016) Magnesium deficiency in plants: an urgent problem. *Crop J* 4:83–91. <https://doi.org/10.1016/j.cj.2015.11.003>
- Gupta AS, Webb RP, Holaday AS, Allen RD (1993) Overexpression of superoxide dismutase protects plants from oxidative stress (induction of ascorbate peroxidase in superoxide dismutase-overexpressing plants. *Plant Physiol* 103:1067–1073. <https://doi.org/10.1104/pp.103.4.1067>
- Haghighi M, Kafi M, Pessarakli M, Sheibanirad A, Sharifinia MR (2016) Using kale (*Brassica oleracea* var. acephala) as a phytoremediation plant species for lead (Pb) and cadmium (Cd) removal in saline soils. *J Plant Nutr* 39:1460–1471. <https://doi.org/10.1080/01904167.2016.1161768>
- Hajer AS, Malibari AA, Al-Zahrani HS, Almaghrabi OA (2006) Responses of three tomato cultivars to sea water salinity 1. Effect of salinity on the seedling growth. *Afr J Biotechnol* 5(10):855–861
- Hameed A, Khan MA (2011) Halophytes: biology and economic potentials. *Karachi Univ Sci* 39:40–44
- Hameed A, Gulzar S, Aziz I, Hussain T, Gul B, Khan MA (2015) Effects of salinity and ascorbic acid on growth, water status and antioxidant system in a perennial halophyte. *AoB Plants*. <https://doi.org/10.1093/aobpla/plv004>
- Howladar SM, Dennett M (2015) Effect of pre-treatment with salicylic acid on vegetative growth and yield components of wheat under salinity. *World Acad Sci Eng Technol Int J Life Sci* 5:9
- Huang Z, Zhao L, Chen D, Liang M, Liu Z, Shao H, Long X (2013) Salt stress encourages proline accumulation by regulating proline biosynthesis and degradation in Jerusalem artichoke plantlets. *PLoS ONE* 8:62085. <https://doi.org/10.1371/journal.pone.0062085>
- Inskeep WP, Bloom PR (1985) Extinction coefficients of chlorophyll a and b in N,N-dimethylformamide and 80% acetone. *Plant Physiol* 77:483–485
- Israeli Y, Lahav E, Nameri N (1986) The effect of salinity and sodium adsorption ratio in the irrigation water, on growth and productivity of bananas under drip irrigation conditions. *Fruits* 41:297–302
- Kumar D, Al Hassan M, Naranjo MA, Agrawal V, Boscaiu M, Vicente O (2017) Effects of salinity and drought on growth, ionic relations, compatible solutes and activation of antioxidant systems in oleander (*Nerium oleander* L.). *PLoS ONE* 12:0185017. <https://doi.org/10.1371/journal.pone.0185017>
- Kumar V, Wani SH, Suprasanna P, Tran LS (eds) (2018) *Salinity responses and tolerance in Plants*, volume 1: targeting sensory, transport and signaling mechanisms. Springer, Berlin
- Kumari A, Das P, Parida AK, Agarwal PK (2015) Proteomics, metabolomics, and ionomics perspectives of salinity tolerance in halophyte. *Front Plant Sci* 6:537. <https://doi.org/10.3389/fpls.2015.00537>
- Küpper H, Andresen E (2016) Mechanisms of metal toxicity in plants. *Metallomics* 8:269–285. <https://doi.org/10.1039/c5mt00244c>
- Levillain J, Cattan P, Colin F, Voltz M, Cabidoche YM (2012) Analysis of environmental and farming factors of soil contamination by a persistent organic pollutant, chlordecone, in a banana production area of French West Indies. *Agr Ecosyst Environ* 159:123–132. <https://doi.org/10.1016/j.agee.2012.07.005>
- Lutts S, Lefèvre I (2015) How can we take advantage of halophyte properties to cope with heavy metal toxicity in salt-affected areas? *Ann Bot* 115:509–528. <https://doi.org/10.1093/aob/mcu264>
- Maathuis FJ (2013) Sodium in plants: perception, signalling, and regulation of sodium fluxes. *J Exp Bot* 65:849–858. <https://doi.org/10.1093/jxb/ert326>
- McElroy JS, Kopsell DA (2009) Physiological role of carotenoids and other antioxidants in plants and application to turfgrass stress management. *N Z J Crop Hortic Sci* 37(4):327–333. <https://doi.org/10.1080/01140671.2009.9687587>
- Muneer S, Kim TH, Choi BC, Lee BS, Lee JH (2014) Effect of CO₂, NO_x and SO₂ on ROS production, photosynthesis and ascorbate-glutathione pathway to Induce *Fragaria* × *annasa* as a hyperaccumulator. *Redox Biol* 1:91–98. <https://doi.org/10.1016/j.redox.2013.12.006>
- Munns R, Tester M (2008) Mechanisms of salinity tolerance. *Annu Rev Plant Biol* 59(1):651–681
- Murali RM, Kumar PD (2015) Implications of sea level rise scenarios on land use/land cover classes of the coastal zones of Cochin, India. *J Environ Manag* 148:124–133. <https://doi.org/10.1016/j.jenvman.2014.06.010>
- Nair (2000) Impacts of climate change and sea level rise on wetlands agriculture: a case study from India. In: *Proceedings of the global forum on salinization and climate change (GFSCC2010)*, Valencia, 25–29 October
- Nawaz MF, Gul S, Tanvir MA, Akhtar J, Chaudary S, Ahmad I (2016) Influence of NaCl-salinity on Pb-uptake behavior and growth of River Red gum tree (*Eucalyptus camaldulensis*

- Dehnh.). Turk J Agric For 40:425–432. <https://doi.org/10.3906/tar-1407-73>
- Nedjimi B (2014) Effects of salinity on growth, membrane permeability and root hydraulic conductivity in three saltbush species. Biochem Syst Ecol 52:4–13. <https://doi.org/10.1016/j.bse.2013.10.007>
- Negrão S, Schmöckel SM, Tester M (2017) Evaluating physiological responses of plants to salinity stress. Ann Bot 119:1–11. <https://doi.org/10.1093/aob/mcw191>
- Pang CH, Wang BS (2008) Oxidative stress and salt tolerance in plants. In: Lüttge U, Beyschlag W, Murata J (eds) Progress in botany. Springer, Berlin, pp 231–245
- Perrier X, De Langhe E, Donohue M, Lentfer C, Vrydaghs L, Bakry F, Carreel F, Hippolyte I, Horry JP, Jenny C, Lebot V (2011) Multidisciplinary perspectives on banana (*Musa* spp.) domestication. Proc Natl Acad Sci USA 108:11311–11318. <https://doi.org/10.1073/pnas.1102001108>
- Piper CS (1950) Soil and plant analysis. Interscience Publishers Inc., New York
- Poustini K, Siosemardeh A, Ranjbar M (2007) Proline accumulation as a response to salt stress in 30 wheat (*Triticum aestivum* L.) cultivars differing in salt tolerance. Genet Resour Crop Evol 54:925–934. <https://doi.org/10.1007/s10722-006-9165-6>
- Qin X, Zhang RX, Ge S, Zhou T, Liang YK (2017) Sphingosine kinase AtSPHK1 functions in fumonisin B1-triggered cell death in Arabidopsis. Plant Physiol Biochem 119:70–80. <https://doi.org/10.1016/j.plaphy.2017.08.008>
- Rahneshan Z, Nasibi F, Moghadam AA (2018) Effects of salinity stress on some growth, physiological, biochemical parameters and nutrients in two pistachio (*Pistacia vera* L.) rootstocks. J Plant Interact 13:73–82. <https://doi.org/10.1080/17429145.2018.1424355>
- Rangani J, Parida AK, Panda A, Kumari A (2016) Coordinated changes in antioxidative enzymes protect the photosynthetic machinery from salinity induced oxidative damage and confer salt tolerance in an extreme halophyte *Salvadora persica* L. Front Plant Sci 7:50. <https://doi.org/10.3389/fpls.2016.00050>
- Rejili M, Vadel AM, Guetet A, Neffatti M (2007) Effect of NaCl on the growth and the ionic balance K⁺/Na⁺ of two populations of *Lotus creticus* (L.) (Papilionaceae). S Afr J Bot 3:623–631. <https://doi.org/10.3389/fpls.2016.00050>
- Robinson JC, Saúco VG (2010) Bananas and plantains, vol 19. Cabi, Wallingford
- Rout NP, Shaw BP (2001) Salt tolerance in aquatic macrophytes: possible involvement of the antioxidative enzymes. Plant Sci 160:415–423. [https://doi.org/10.1016/S0168-9452\(00\)00406-4](https://doi.org/10.1016/S0168-9452(00)00406-4)
- Sagisaka S (1976) The occurrence of peroxide in a perennial plant, *Populus gelrica*. Plant Physiol 57:308–309. <https://doi.org/10.1104/pp.57.2.308>
- Shapira OR, Khadka S, Israeli Y, Shani UR, Schwartz A (2009) Functional anatomy controls ion distribution in banana leaves: significance of Na⁺ seclusion at the leaf margins. Plant, Cell Environ 32(5):476–485. <https://doi.org/10.1111/j.1365-3040.2009.01941>
- Simões WL, Calgaro M, Coelho DS, Santos DB, Souza MA (2016) Growth of sugar cane varieties under salinity. Rev Ceres 63(2):265–271. <https://doi.org/10.1590/0034-737X201663020019>
- Smith IK, Vierheller TL, Thorne CA (1989) Properties and functions of glutathione reductase in plants. Physiol Plant 77:449–456. <https://doi.org/10.1111/j.1399-3054.1989.tb05666.x>
- Sohail M, Saied AS, Gebauer J, Buerkert A (2009) Effect of NaCl salinity on growth and mineral composition of *Ziziphus spina-christi* (L.) Willd. J Agric Rural Dev Trop 110:107–114
- Taïbi K, Taïbi F, Abderrahim LA, Ennajah A, Belkhdja M, Mulet JM (2016) Effect of salt stress on growth, chlorophyll content, lipid peroxidation and antioxidant defence systems in *Phaseolus vulgaris* L. S Afr J Bot 105:306–312. <https://doi.org/10.1016/j.sajb.2016.03.011>
- Tewari RK, Kumar P, Tewari N, Srivastava S, Sharma PN (2004) Macronutrient deficiencies and differential antioxidant responses—influence on the activity and expression of superoxide dismutase in maize. Plant Sci 166:687–694. <https://doi.org/10.1016/j.plantsci.2003.11.004>
- Tripathi DK, Singh VP, Chauhan DK, Prasad SM, Dubey NK (2014) Role of macronutrients in plant growth and acclimation: recent advances and future prospective. In: Ahmad P, Wani M, Azooz M, Phan Tran LS (eds) Improvement of crops in the era of climatic changes 2014. Springer, New York, pp 197–216
- Turhan A, Kescu H, Ozmen N, Sitki Serbeci M, Osman Demir A (2014) Effect of different concentrations of diluted seawater on yield and quality of lettuce. Chil J Agric Res 74:111–116. <https://doi.org/10.4067/S0718-58392014000100017>
- Van den Brand TP, Roest K, Chen GH, Brdjanovic D, van Loosdrecht MC (2015) Effects of chemical oxygen demand, nutrients and salinity on sulfate-reducing bacteria. Environ Eng Sci 32:858–864. <https://doi.org/10.1089/ees.2014.0307>
- Vilardy SP, González JA, Martín-López B, Montes C (2011) Relationships between hydrological regime and ecosystem services supply in a Caribbean coastal wetland: a social-ecological approach. Hydrolog Sci J 56:1423–1435. <https://doi.org/10.1080/02626667.2011.631497>
- White PJ, Broadley MR (2003) Calcium in plants. Ann Bot 92:487–511. <https://doi.org/10.1093/aob/mcg164>
- Willadino L, Camara TR, Ribeiro MB, Amaral DO, Suassuna F, Silva MV (2017) Mechanisms of tolerance to salinity in banana: physiological, biochemical, and molecular aspects. Rev Bras Frutic. <https://doi.org/10.1590/0100-29452017723>
- Yano-Melo AM, Saggini OJ Jr, Maia LC (2003) Tolerance of mycorrhized banana (*Musa* sp. cv. Pacovan) plantlets to saline stress. Agric Ecosyst Environ 95:343–348. [https://doi.org/10.1016/S0167-8809\(02\)00044-0](https://doi.org/10.1016/S0167-8809(02)00044-0)
- Zhang M, Fang Y, Ji Y, Jiang Z, Wang L (2013) Effects of salt stress on ion content, antioxidant enzymes and protein profile in different tissues of *Broussonetia papyrifera*. S Afr J Bot 85:1–9. <https://doi.org/10.1016/j.sajb.2012.11.005>
- Zushi K, Matsuzoe N (2017) Using of chlorophyll a fluorescence OJIP transients for sensing salt stress in the leaves and fruits of tomato. Sci Hortic 219:216–221. <https://doi.org/10.1016/j.scienta.2017.03.016>

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