



Full Length Article

Physiological Responses of the Halophyte *Salvadora persica* to the Combined Effect of Salinity and Flooding

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Abstract

Miswak (*Salvadora persica* L.) is a subtropical shrub common in habitats with fluctuating soil salinity and sporadic floods. Little is known about its response to the combination of salinity and flooding (hypoxia) stresses. This study is an attempt to characterize the physiological response of Miswak to the combination of these two stresses *in situ*. We examined functional leaf traits, osmotic adjustment, gas exchange rates, chlorophyll a fluorescence and lipid peroxidation. Under stressful conditions, this species was able to shift from fast to slow growth and adjust its gas exchange rates in order to reduce water loss and limit the upward transport of toxic ions. In saline-dry(S) and saline-flooded (S-F) habitats stomatal conductance (g_s) and stomatal density (SD) decreased as Na accumulated in the leaves. In contrast, in non-saline-flooded (NS-F) habitats SD increased. Na^+ and K^+ contributed greatly to leaf osmotic potential (Ψ_π), especially under the combined effects of salinity and flooding. Proline concentrations increased too in root and leaf tissues possibility to protect macromolecules against the rising Na^+ concentration. Furthermore, photorespiration and cyclic electron transport appear to have been upregulated in non-saline-flooded habitats and appear to play a protective role. However under prolonged saline stress, thermal dissipation was involved in PSII protection but not photo-inhibition. Still under saline and saline-flooded habitats, plants were able to reduce the flow of electrons through photosystems. Under saline-flooded habitats plants experience oxidative stress leading to photo-damage. A combination of morphological (smaller leaves) and physiological (accumulation of Na^+ and K^+ in the leaves) adjustments and increased osmo- (accumulation of proline) and photo- (reduction in chlorophyll content, increased non-photochemical quenching and reduced Photochemical quenching (q_p) and linear electron transport rate) protection helped the plant overcome salinity, hypoxia and their combination in its native habitats. © 2018 Friends Science Publishers

Keywords: Saline-flooded habitats; Osmotic adjustment; Soluble sugars; Proline; Chlorophyll fluorescence

Introduction

Saudi Arabia (SA) is considered one of the driest countries in the world. The aridity index classifies the country as having desert conditions with extreme heat during the day, and little, and irregular precipitation (Dabbagh and Abderrahman, 1997). Still the southwestern part of the country (Tihama and Asir regions) is considered semiarid receiving the highest rainfall in the country. Sporadically, there can be heavy rain storms causing serious flash floods in the coastal lowlands of Tihama (El-Demerdash *et al.*, 1995; Dabbagh and Abderrahman, 1997). Large scale urban development projects in recent decades have raised the risk of floods with serious consequences for plant communities in the wadis (riparian) and coastal saline ecosystems (estuarine) (Voss *et al.*, 2013; Ge *et al.*, 2016; Li *et al.*, 2018). Therefore, understanding the effect of flooding (hypoxia) as well as the combined effect of flooding and soil salinity on the responses of vegetation is needed to

manage the sea-level rise and storms effects due to climate change (Barrett-Lennard and Shabala, 2013; Li *et al.*, 2018). Still the general effects of salinity (Tounekti *et al.*, 2012) and flooding stress (Voss *et al.*, 2013; Di Bella *et al.*, 2015) on many plant species are well documented, but the simultaneous effects of flooding and salinity are not fully understood (Gorai *et al.*, 2010; Barrett-Lennard and Shabala, 2013; Li *et al.*, 2018). Nowadays such combined effects are considered as important factors that cause the absence of many plant species in several salt-affected soils; hence they are needed to be considered when replanted the saline areas (El-Demerdash *et al.*, 1995; Herrera, 2013; Voss *et al.*, 2013; Li *et al.*, 2018). Previous reports showed a decrease in the growth rate of several halophytes during extended flooding, though such effect on growth may vary among species (Voss *et al.*, 2013). In addition to the osmotic and the toxic effects of the salinity, the inhibition of aerobic root respiration (hypoxia) may inhibit the uptake and transport of nutrients in the plant as well affect the exclusion

of toxic ions like Na^+ and Cl^- from roots and leaves (Barrett-Lennard and Shabala, 2013; Shabala and Pottosin, 2014). The hypoxic conditions can affect the hormonal balance and photosynthetic machinery (Colmer and Voesenek, 2009; Gorai *et al.*, 2010) and reduce ATP formation. Such conditions could affect the growth and survival of roots and reduce the uptake of several essential ions (Barrett-Lennard and Shabala, 2013). Plants' demand for O_2 can be reduced by mechanisms such as low respiration activity, increased antioxidant capacity and the development of adventitious roots holding aerenchyma that aids the plant to increase water and nutrient absorption under hypoxia (Di Bella *et al.*, 2015). So, in this paper, we considered a set of functional leaf traits and physiological parameters to better appreciate the response of the halophyte *S. persica* L. (Salvadoraceae) to salinity and flooding stresses.

S. persica, commonly named Miswak, is a facultative halophyte (Maggio *et al.*, 2000; Rao *et al.*, 2004; Rangani *et al.*, 2016) that can grow in several hostile ecological systems ranging from non-saline to highly-saline soil, and dry regions to marshy and waterlogged areas (Zodape and Indusekhar, 1997; Reddy *et al.*, 2008). It occupies the open mangrove patches as well as on the high tidal areas mudflats. It may occur as islands of woodland in the middle of these mudflats. In these saline areas, inundating events take place after a long last rainfall. In some coastal plains, *S. persica* is located at downstream (deltas) of major Wadis and water abundance in these areas allows the stagnation of the water for a long period. Miswak is also prevalent in the beds of the wadis and near wadis and lakes (Al-Jaloud *et al.*, 2001).

Studies on the biology, physiology and on the medicinal usage of Miswak were previously reviewed (Reddy *et al.*, 2008). It was shown that *S. persica* species are potentially interesting for the food and cosmetic industries (Ahmad and Rajagopal, 2013). The seed oil of *S. persica* is a good source of auric acid and contains 40–50% fat used in soap and detergent industries (Ahmad and Rajagopal, 2013). Its roots are regularly used as tooth brush and constitute a good source of some pharmaceutical derivatives (WHO, 1987). Since it grows both inland and in coastal wetland, *S. persica* has high ecological value in arid and semiarid ecosystem (Al-Jaloud *et al.*, 2001). It is recommended for the reclamation of both sand dunes and saline soils habitats. However, the present stands in Tahama plains of south-western Saudi Arabia are under considerable pressure for over-grazing and uprooting. Hence, there is need to protect certain areas where these species is well adapted to allow it to regenerate. Furthermore, other areas where the stands were degraded need to be replanted. To do so, we have to develop a good understanding of the ecophysiology of the species especially how it adapts to various edaphic conditions (Gorai *et al.*, 2010; Voss *et al.*, 2013). The changes in precipitation frequency and distribution and sea level due to climate change further justify a closer look at the species behaviour under

particular environmental conditions such as flooding and soil salinity (Voss *et al.*, 2013; Ge *et al.*, 2016; Li *et al.*, 2018). This study evaluated the effects of salinity and flooding (hypoxia) on the physiology of natural stands of *S. persica*. The species ability to overcome simultaneous stresses was assessed by evaluating several ecophysiological attributes including functional leaf traits, stomatal density, osmotic adjustment mechanisms, leaf gas exchange, chlorophyll (Chl) fluorescence, lipid peroxidation, as well as proline and TSS contents.

Materials and Methods

Study Area and Plant Material

Jazan province is situated in the south-western part of Saudi Arabia near the Saudi/Yemeni border between longitudes 42° and 43,8°E and latitudes 16,5° and 17°N (Fig. 1). The region has a subtropical climate, where the average annual temperature is 23°C and the average annual rainfall varies from 100 on the coast (the study area) to 500 mm in the mountains (Dabbagh and Abderrahman, 1997). The coastal areas of Jazan are frequently subjected to flash floods due to heavy downpours on the mountains. From a biogeographical point of view, the vegetation of this region is closely related to that of East Africa (El-Demerdash *et al.*, 1995). The halophytic community of *S. persica*, *Suaeda monoica* and *Tamarix aphylla* and *Cyperus conglomeratus* dominates the holomorphic and hydromorphic soils of the sabkhas (Al-Jaloud *et al.*, 2001). Interestingly *S. persica* can also grow along wadis and estuaries in the drier sites to the east up to about 50 km from the sea shores. Moreover, it is prevalent in coastal (intertidal and abovetidal regions) and inland saline habitats (sabkha), near the foot-hills up to an altitude of 900 m near Addayer and Maraba and in the beds of wadis as well as on Tihama flood plains (Sabya, Abu-Arish, Madaya, Samtah and Al-Muwassem) (El-Demerdash *et al.*, 1995).

A number of *S. persica* stands (identified by Dr. R. R. Mochikkal from the Jazan University Herbarium, Saudi Arabia) were selected randomly for sampling and measurements during the period of October and November 2015 when most of the coastal area has been flooded earlier in September (Table 1 and Fig. 1). Moreover, at this time of the year, large water ponds remained for several months in the bed of wadi Jazan submerging the vegetation for prolonged period of time. The sabkhas of Madaya and Sabya are frequently subjected to flush flooding after heavy rain on the mountains. These sabkhas are commonly saturated with brine and their shores are colonized by several halophytes including *S. persica* (Al-Jaloud *et al.*, 2001). The salinity of the sabkha soil varies from region to region and from season to season; it decreases considerably due to flooding and increases during the dry season (May-July). The soil's ECe ranges from 19.5 to 25.7 dSm⁻¹ and its texture varies from coarse (sandy) to fine (sandy-clay-loam) depending on the sampling site.

Table 1: Soil characteristics of the four sites used to study the adaptation of *S. persica* to salinity and flooding. Leaf area (L_A), specific leaf area (SLA), leaf mass area (LMA), succulence (S), leaf dry matter content (LDMC), leaf thickness (L_{th}), leaf water content (LWC), stomatal density (SD) of *S. persica* plants growing in diverse habitats in Jazan region of Saudi Arabia. The values are means $\pm$ SE of at least six replicates

Plant habitat	Non-saline habitats (Wadis)	Non-saline flooded habitats	Saline-dry habitats (Unflooded Sabkhas)	Saline-flooded habitats (Flooded sabkhas)
SWC (%)	6.7 $\pm$ 0.6	33.5 $\pm$ 3.5	4.6 $\pm$ 0.8	24.8 $\pm$ 2.9
Soil ECe (dS m ⁻¹)	4.4 $\pm$ 1.4	2.27 $\pm$ 0.7	25.7 $\pm$ 5.3	23.3 $\pm$ 3.1
Soil pH	7.1 $\pm$ 0.2	7.3 $\pm$ 0.3	8.5 $\pm$ 0.1	8.4 $\pm$ 0.1
L_A (cm ²)	11.73 $\pm$ 0.67	16.05 $\pm$ 0.85	8.61 $\pm$ 0.62	13.54 $\pm$ 0.80
LWC (g g ⁻¹ Dw)	4.27 $\pm$ 0.19	3.61 $\pm$ 0.20	2.07 $\pm$ 0.09	3.05 $\pm$ 0.15
SLA (m ² kg ⁻¹ Dw)	10.83 $\pm$ 0.54	9.67 $\pm$ 0.59	5.43 $\pm$ 0.28	6.06 $\pm$ 0.33
LMA (g mm ⁻²)	0.96 $\pm$ 0.05	1.15 $\pm$ 0.07	1.95 $\pm$ 0.11	1.73 $\pm$ 0.09
S (mg H ₂ O cm ⁻²)	40.18 $\pm$ 1.76	38.60 $\pm$ 1.86	39.82 $\pm$ 2.03	51.56 $\pm$ 2.48
LDMC (g g ⁻¹)	0.19 $\pm$ 0.00	0.23 $\pm$ 0.01	0.33 $\pm$ 0.00	0.25 $\pm$ 0.01
L_{th} (mm)	0.50 $\pm$ 0.01	0.50 $\pm$ 0.02 $\pm$	0.59 $\pm$ 0.02	0.69 $\pm$ 0.02
SD on adaxial surface (Number mm ⁻²)	40 $\pm$ 4	49 $\pm$ 3	34 $\pm$ 5	15 $\pm$ 7
SD on abaxial surface (Number mm ⁻²)	49 $\pm$ 5	61 $\pm$ 8	41 $\pm$ 4	18 $\pm$ 2

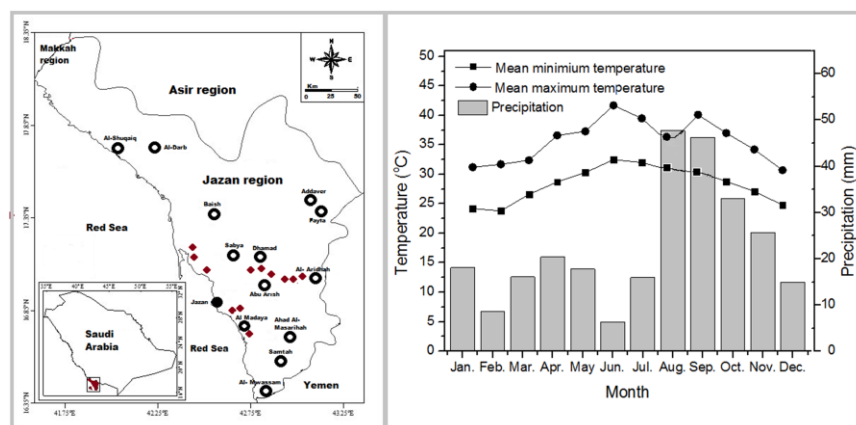


Fig. 1: Location map showing sampling sites in Jazan province, Saudi Arabia. The graph to right shows mean maximum temperature, mean minimum temperature, monthly sum of precipitation in Jazan during the study period (2015)

In total, four sites were selected: (i) non-saline-dry site (NS, dry wadi, south of Dhamad), (ii) flooded–non-saline site (NS-F, flooded wadis and floodplains, west of Abu-Arish), (iii) saline-dry site (S, Dry sabkha, Sabya) and (iv) flooded-saline site (S-F, flooded sabkha, Madaya). At each site, eight to twelve bushes of *S. persica* were tagged for sampling. Canopy diameter ranged from four to six meters. Healthy-looking, fully expanded leaves, stems, and roots were collected, transported to the laboratory in a cool box, dipped in liquid nitrogen then stored in a -80°C freezer until needed. Another set of samples of the same plant organs were collected then oven-dried for the determination of ions and leaf water content.

Soil Water Content and Salinity Measurements

From each site, soil samples (5 kg) were taken from the area adjacent to tree roots. The samples were air dried in the laboratory the stored for subsequent use. For electrical conductivity, the soil was extracted in deionized water (1:1, w:v). Soil salinity was considered ‘moderate’ for ECe from

2 to 6 dS m⁻¹, ‘high’ for ECe between 6 and 15 dS m⁻¹ and ‘extreme’ for ECe over 15 dS m⁻¹ (Richards, 1954). Soil water content (SWC) was measured by the gravimetric method (Black, 1965).

Functional Leaf Traits Analysis and Plant Water Relations

Aiming to assess the morphological adaptations used by *S. persica* to endure contrasting environmental conditions, several functional leaf traits such as leaf mass area (LMA), leaf succulence (S), leaf dry matter content (LDMC), specific leaf area (SLA), and leaf water content (LWC) were estimated. LWC was calculated as follow: $LWC = (L_{FW} - L_{DW}) / L_{FW}$ with L_{DW} as leaf dry weight (g) and L_{FW} as leaf fresh weight (g). L_{DW} is the dry weight after oven-drying the leaves at 80°C for 48 h. Leaf area (L_A) was measured using a LI-3000C Portable Area Meter (LI-COR Biosciences, Lincoln, Nebraska, USA) and the LMA was calculated as the ratio of LDMC/ L_A . SLA was calculated as $SLA = L_A / L_{DW}$ (Wright *et al.*, 2004). The LDMC was

estimated as $LDMC = L_{DW}/L_{FW}$ and the leaf density (L_D) was considered approximately equal to LDMC. Leaf thickness (L_{th}) or leaf succulence (S) were determined as $L_{th}=S = L_{FW}/L_A$ (Poorter and Garnier, 2007).

Plant water potential (Ψ_w) was measured with a pressure chamber (PMS, Albany, OR, USA) on shoots collected in the early morning before sunrise. Part of these shoots was frozen upon arrival to the laboratory and their osmotic potentials (Ψ_s) were determined using a Wescor 5500 vapour pressure osmometer (Wescor Inc., Logan, UT, USA). The Van't Hoff relationship was used to determine the ion contribution to osmotic potential (Ψ_π) as follow: solute potential = $-C \times R \times T$, with C = molarity of the solution (mol of solute kg^{-1} H_2O), R = gas constant (0.00831 $kg\ mol^{-1}\ K^{-1}$) and T = temperature (Debez *et al.*, 2004).

Ion Contents and Selective Transport Capacity for K^+ over Na^+

Dried leaves, stems and roots were extracted in $0.1\ M\ HNO_3$ for determination of their K^+ and Na^+ contents using a PFP7 flame photometer (Jenway, UK). Selective transport capacity (ST) for K^+ over Na^+ between the organs was determined as follow: $ST_{(A/B)} = (Na^+/K^+ \text{ in part A}) / (Na^+/K^+ \text{ in part B})$ (Debez *et al.*, 2004).

Gas Exchange and Chlorophyll *a* Fluorescence Measurements

Net photosynthetic assimilation rate (A), stomatal conductance (g_s) and transpiration rate (E) were measured on fully expanded leaves on actively growing shoots from the median part of the canopy facing east using a CIRAS 2 infra-red gas analyser (PP Systems, USA). Instantaneous water use efficiency ($iWUE$) was calculated as: $iWUE = A/E$ (Debez *et al.*, 2004).

Pulse modulated chlorophyll fluorescence meter (FMS2, Hansatech Instruments Ltd, UK) was used to measure chlorophyll *a* (Chl) fluorescence. A modulated light ($<0.1\ \mu mol\ photon\ m^{-2}\ s^{-1}$) was applied to dark adapted- (30 min) leaves to determination the minimal fluorescence (F_0) value, while the maximal fluorescence (F_m) value was obtained by application of a saturating light pulse ($3900\ \mu mol\ quanta\ m^{-2}\ s^{-1}$; pulse duration: 0.8s). F_0 and F_m values were used to calculate maximum photochemical quantum yield of PSII ($F_v/F_m = F_m - F_0/F_m$). Afterward, these light-adapted leaves were continually irradiated with actinic light to measure the steady-state (F_s), maximal fluorescence (F_m') and the minimal fluorescence (F_0'). Effective photochemical quantum yield of PSII was calculated as $F_m' - F_s/F_m'$ (Baker, 2008). Non-photochemical quenching of fluorescence (NPQ) was calculated as $NPQ = F_m/F_m' - 1$. The coefficient of photochemical quenching (qP) was calculated as $(F_m' - F_s)/(F_m' - F_0')$ (Baker, 2008). The linear electron transport rate (ETR) was calculated as $ETR = (\Delta F/F_m') \times PPFD \times 0.5 \times 0.84$ where PPFD is the photosynthetic photon flux density (Baker, 2008).

Leaf Chlorophyll Content Determination

Chlorophylls (Chl *a* and *b*) and carotenoids were extracted from frozen leaves using 100% acetone and quantified spectrophotometrically using the method of Arnon (1949).

Total Soluble Sugar (TSS) and Proline Contents

TSS were determined spectrophotometrically using the anthrone method (Yemm and Willis, 1954). Proline content in $0.2\ g$ of frozen leaves was determined using the ninhydrin method (Bates *et al.*, 1973).

Malondialdehyde Content

Malondialdehyde (MDA) contents were determined spectrophotometrically using the method of Hodges *et al.* (1999).

Statistical Analyses

The GLM procedure of SAS software (SAS, 1996) was used to analyse the variance of the data. Where applicable, the means were separated with Duncan Multiple Range Test ($P \leq 0.05$). The number of replicates varied from eight to twelve.

Results

Leaf Functional Traits

Specific leaf area (SLA), L_{th} , LDMC, S and LMA were used in this study as indicators on plant growth and leaf adaptation strategies (Table 1). Trees of flooded areas had larger leaves than trees of dry sites; L_A was 12% larger (Table 1). In contrast, salinity of the medium (ECe between 19.5 and $25.7\ dS\ m^{-1}$) significantly decreased (27%) the L_A compared to trees in non-saline sites.

The SLA decreased while LMA and LDMC (or leaf density) increased for *S. persica* plants growing either under saline-dry (sabhka, S) or saline-flooded habitats (S-F). Flooding stress did not affect significantly SLA of the populations growing in saline and non-saline habitats. The leaf thickness (L_{th}) and LDMC of *S. persica* increased under saline-dry habitats, and flooding stress increased further these two parameters. Furthermore the leaf succulence index (S) increased only under the combined effect of salinity and flooding.

The Table 1 show also that flooding stress have increased the stomata density (SD) of Miswak plants grown under non-saline habitats. In contrast, under saline-dry and saline-flooded habitats, the SD was reduced. The SD was significantly higher in the adaxial surface of the leaves compared to the abaxial surface in all the plant habitats either flooding or salinity.

Plant Water Relations

Our results show that either flooding stress, salinity or their combination decreased leaf water content (LWC) (Table 1). Still LWC was lowest under saline-dry medium of the sabkha. Plant Ψ_w and Ψ_π decreased considerably due to soil salinity of sites S and S-F (Fig. 2). Shoot Ψ_w and Ψ_π were lowest (-2.3 and -3.2 MPa respectively) in the trees growing the saline sabkha. Flooding increased slightly the Ψ_w and Ψ_p of the plants growing in the Sabkha. However, the Ψ_w and Ψ_π did not change in the trees growing in non-saline sites.

Gas Exchange

Our results revealed that g_s and E decreased under flooding (Fig. 3). The increases of the A/g_s ratio under such conditions indicate that photosynthesis was inhibited by stomatal closure. Besides, salinity of the medium decreased all gas exchange parameters including A , g_s , and E whereas $iWUE$ was increased (Fig. 2).

Mineral Nutrition and Osmotic Adjustment

Plants growing in saline environments, flooded or not, accumulated high concentrations of Na^+ but low amounts of K^+ in their leaves, stems and roots (Fig. 4-A). The leaves had the highest concentrations of Na^+ among the three tissues. In particular, plants growing in sabkhas had almost 20 times more Na^+ in their leaves than those growing in non-saline sites. Flooding further increased the concentrations of Na^+ in the leaves and shoots. In contrast, soil salinity considerably reduced K^+ concentration (-34%) and K^+/Na^+ ratio (-35%) in the leaves as compared to the plants living in non-saline sites. Interestingly, our results indicate that the capacity to transport K^+ over Na^+ from the roots to the stems ($ST_{(root/stem)}$) was not significantly affected by salinity or flooding of the area Miswak (Fig. 5); whereas, Miswak transport of the toxic cation (Na^+) over K^+ from the stems to the leaves was considerably increased (lower $ST_{(stem/leaf)}$ values) when the site was saline.

Proline, Total Soluble Sugars and Contribution to the Osmotic Potential

Miswak plants growing in saline-dry habitats accumulated five times more proline but half as much TSS than plants growing in non-saline habitats (Fig. 4-B). Flooding tended to further increase leaf and root proline concentrations and reduce TSS concentrations compared to the plants growing in saline-dry habitats.

Na^+ and K^+ contribute around 40% of the Ψ_π of the trees growing in the saline-dry site (sabkha) (Fig. 5). Still the combined effect of salinity and flooding further increased this contribution to 69%. The contribution of proline and TSS was only about 5% under saline-dry habitats. Flooding increased the contribution of proline but decreased the contribution of TSS in the plants growing in saline-dry habitats (Fig. 5).

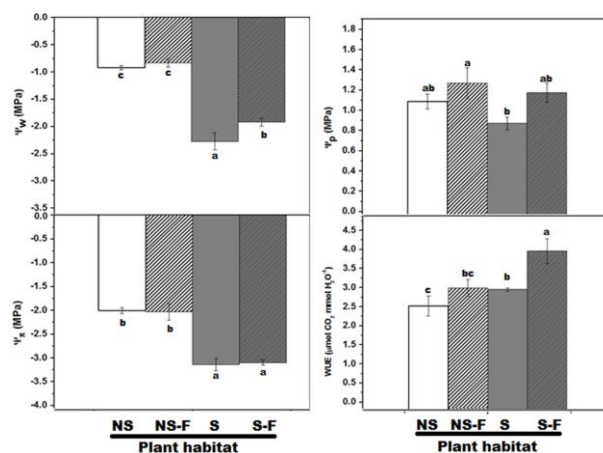


Fig. 2: Water potential (Ψ_w), osmotic potential (Ψ_π), turgor potential (Ψ_p), and instantaneous water use efficiency ($iWUE$) of *S. persica* plants growing in diverse habitats in Jazan region. NS: Non-saline sites, NS-F: non-saline-flooded sites, S: saline-dry sites, S-F: saline-flooded sites. The results are means $\pm$ SE of at least six replicates; different letters indicate significant differences at $P < 0.05$ in response to changes in plant habitat (Duncan's test)

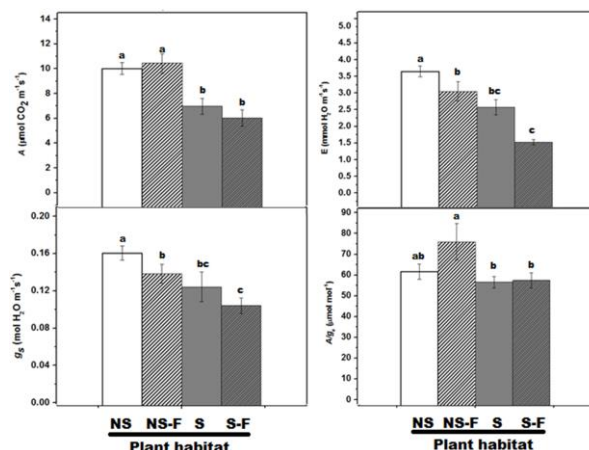


Fig. 3: Net photosynthetic assimilation rate (A), stomatal conductance (g_s), transpiration rate (E) and A/g_s ratio of *S. persica* plants living in diverse habitats in Jazan region. NS: Non-saline habitats, NS-F: non-saline-flooded habitats, S: saline-dry habitats, S-F: saline-flooded habitats. The results are means $\pm$ SE of at least six replicates; different letters indicate significant differences at $P < 0.05$ in response to changes in plant habitat (Duncan test)

Photosynthetic Pigments and Chl *a* Fluorescence

Flooding alone did not affect the photosynthetic pigments or carotenoids of *S. persica* leaves (Fig. 6); whereas, salinity or the combination of salinity and flooding reduced the concentrations of all three pigments. The Chl *a/b* ratio increased due to salinity and flooding compared to non-saline dry situation.

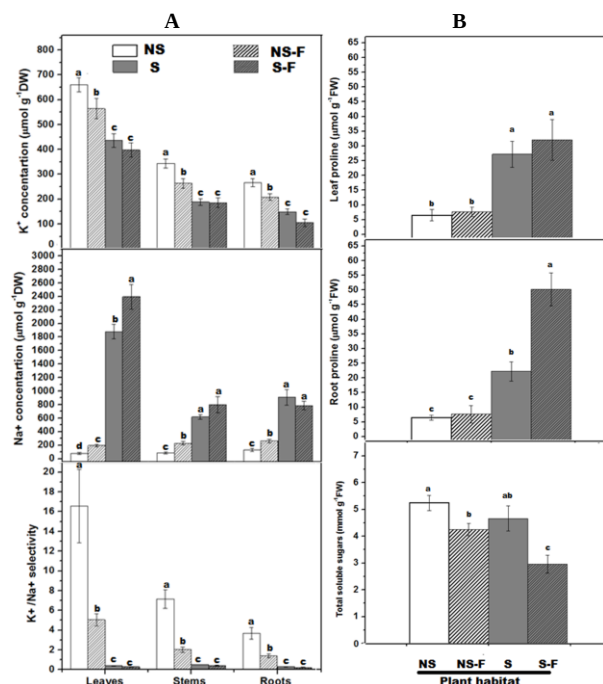


Fig. 4: (A) Accumulation of Na⁺ and K⁺ and selectivity K⁺/Na⁺ in different organs of *S. persica* plants growing in different habitats in Jazan region. NS: non-saline habitats, NS-F: non-saline-flooded habitats, S: saline-dry habitats, S-F: saline-flooded habitats. The results are means $\pm$ SE of at least six replicates; different letters indicate significant differences at $P < 0.05$ in response to changes in plant habitat (Duncan test). (B) Leaf proline, root proline and total soluble sugars (TSS) concentrations in *S. persica* plants growing in diverse habitats in Jazan region. NS: non-saline habitats, NS-F: non-saline-flooded habitats, S: saline-dry habitats, S-F: saline-flooded habitats. Results are means $\pm$ SE of six replicates; different letters indicate significant differences at $P < 0.05$ in response to changes in plant habitat (Duncan test)

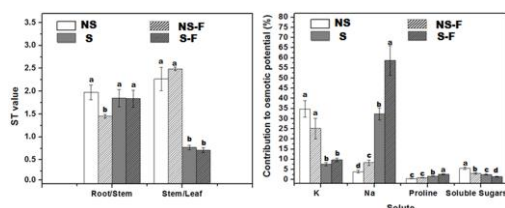


Fig. 5: Selectivity of transport K⁺ over Na⁺ (ST) from roots to stems (ST_{root/stem}) and from stems to leaves (ST_{stem/leaf}) and the contribution of K⁺, Na⁺, proline and soluble sugars to osmotic potential of *S. persica* plants growing in diverse habitats in Jazan region. NS: Non-saline habitats, NS-F: non-saline-flooded habitats, S: saline-dry habitats, S-F: saline-flooded habitats. Results are means $\pm$ SE of at least six replicates; different letters indicate significant differences at $P < 0.05$ in response to changes in plant habitat (Duncan test)

According to our results, photo-protective mechanisms were adjusted according to the prevalent stress (Fig. 7). For instance, *S. persica* plants growing under non saline-flooded habitats did not increase the non-photochemical quenching (NPQ) nor significantly decreased maximum quantum efficiency of PSII (F_v/F_m). Still under saline-dry habitats (sabkha), F_v/F_m values were always higher than 0.7. However, in saline-flooded sites, F_v/F_m ratio decreased to about 0.6 indicating the occurrence of photo inhibition despite the apparent increase in NPQ. Photochemical quenching (q_p), effective photochemical efficiency (Φ_{PSII}) and linear electron transport rate (ETR) decreased in response to salinity and the combination of salinity and flooding.

Lipid Peroxidation

The MDA content (lipid peroxidation level) increased slightly due to salinity of the sabkha (Fig. 6), whereas, flooding did not significantly alter MDA content in plant tissues.

Discussion

Salvadora persica is a facultative halophyte which is adapted to contrasting environmental conditions ranging from non-saline to highly saline soils and from dry to marshy and flooded areas (Zodape and Indusekhar, 1997; Reddy et al., 2008). Previous studies have shown that *S. persica* can tolerate salt concentrations close to that of seawater (Rao et al., 2004). It was grown with up to 250 mM NaCl in the culture medium with no significant adverse effects on its growth (Rangani et al., 2016). The decrease in leaf expansion is one of the first responses of plants to several abiotic stresses such salinity (Tounekti et al., 2012; Osnas et al., 2013). In the present study, the average leaf area (L_A) of *S. persica* trees growing in saline-dry sites (ECe between 19.5 and 25.7 dS m⁻¹) was 27% smaller than that of trees growing in non-saline sites. Such reduction in leaf area can save energy, minimize the production of toxic reactive oxygen species (ROS) by reducing the flux of light intercepted by the antennas of photosystems thus limiting the excitation energy load, and provide more free amino acids for osmotic adjustment (Debez et al., 2008; Flowers et al., 2015). Furthermore, the decrease of L_A limits water loss by transpiration and thus reduces the transport of toxic ions from the roots to the shoots (Barrett-Lennard and Shabala, 2013). Similarly, flooding impairs L_A expansion too mainly due to a reduction in nutrient uptake (Barrett-Lennard and Shabala, 2013). In the present study, flooding actually increased the L_A of Miswak plants suggesting that the roots of this species are fairly tolerant to hypoxia.

The examination of several functional leaf traits (Table 1) reveal that *S. persica* adapted well to salinity, flooding or their combination. The SLA, LMA, LDMC, L_{th} and S have been used in this study as indicators of plant growth and leaf

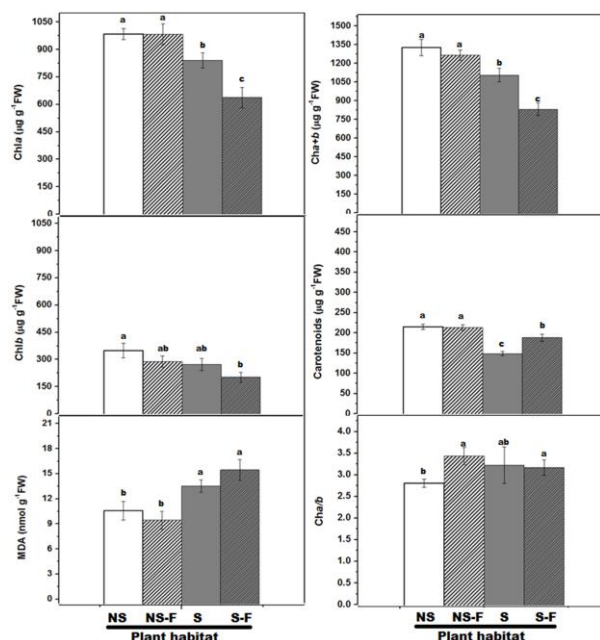


Fig. 6: Chlorophyll a (Chl a) and Chlorophyll b (Chl b) and total chlorophylls (Chl a+b), chl a/b ratio, carotenoids and peroxidation of lipids (MDA) of *S. persica* plants growing in diverse habitats in Jazan region. NS: non-saline habitats, NS-F: non-saline-flooded habitats, S: saline-dry habitats, S-F: saline-flooded habitats. The results are means $\pm$ SE of at least six replicates; different letters indicate significant differences at $P < 0.05$ in response to changes in plant habitat (Duncan test)

adaptation strategies (Wright *et al.*, 2004; Osnas *et al.*, 2013). SLA is highly correlated with A_{net} and plant growth rate (Wright *et al.*, 2004). Like in many other halophytes (Poorter and Garnier, 2007), SLA decreased (or LMA increased) while LDMC (or L_D) increased for miswak plants growing either in saline-dry (sabkha) or saline-flooded habitats. The decline of SLA due to flooding seems to make the mesophyll parenchyma less compact thus facilitating O_2 diffusion into the mesophyll (Herrera, 2013). Besides, lower SLA values contribute to leaf longevity, nutrient retention, and protection from dehydration (Wright *et al.*, 2004). As in many halophytes (Debez *et al.*, 2008), the L_{th} and LDMC (L_D) of *S. persica* increased under saline conditions. This agrees with reports on *Alchornea triplinervia*, in which flooding increased the thickness of palisade and spongy parenchyma by 1.4 folds (Roças and Scarano, 2001). Furthermore, leaves became more succulent (S increased) when the trees were subjected to both salinity and flooding. Such response is considered an adaptation in order to save water and dilute toxic ions in halophytes (Koyro *et al.*, 2013).

Generally, the anatomical changes which occur in response to stress are designed to optimize leaf gas-exchange (Shabala and Pottosin, 2014). In fact, our results

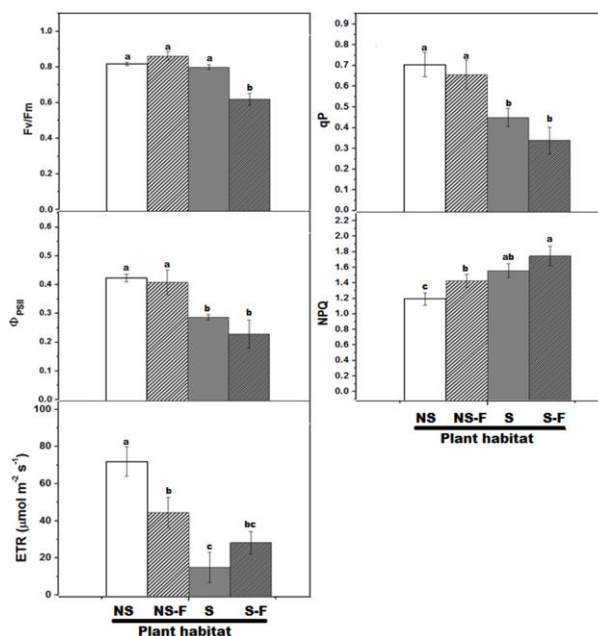


Fig. 7: Leaf chlorophyll a fluorescence parameters of *S. persica* plants growing in diverse habitats in Jazan region. NS: non-saline habitats, NS-F: non-saline-flooded habitats, S: saline-dry habitats, S-F: saline-flooded habitats. The results are means $\pm$ SE of at least six replicates; different letters indicate significant differences at $P < 0.05$ in response to changes in plant habitat (Duncan test)

show that transient flooding of Miswak plants grown in non-saline habitats led to an increase of stomata density (SD) indicating an increased demand of CO_2 (Shabala and Pottosin, 2014). In contrast, in saline-dry and saline-flooded habitats, SD decreased possibly to reduced water loss by transpiration.

The detrimental effects of salinity on halophytes and glycophytes are generally due to osmotic effects and/or ionic imbalances as a result of nutrients deficiencies or ions toxicities (Debez *et al.*, 2008). For instance, when plants fail to hydrate their tissues properly on saline soils they suffer from physiological drought. To maintain a suitable cell turgor in their leaves, halophytes accumulate high amounts of solutes, mainly Na^+ , Cl^- and K^+ , in their vacuoles (Flowers *et al.*, 2015). In the present study, *S. persica* was able to adequately lower its Ψ_w and Ψ_π in the saline-dry sabkha, similar to what generally happens in other halophytes (Maggio *et al.*, 2000; Shabala and Pottosin, 2014). Shoot Ψ_w reached its lowest values (down to -2.3 MPa) in the sabkha indicating that the trees were capable of osmotically adjust under both saline-dry and saline-flooded conditions (Flowers *et al.*, 2015). This aided salt-stressed Miswak plants to preserve leaf cell turgor as indicated by positive Ψ_p values. Flooding increased slightly the Ψ_w and Ψ_π of the plants growing in the sabkha allowing them to

maintain their Ψ_p . In non-saline-flooded habitats, tree Ψ_w did not change and little changes in LWC were seen, which suggests that flooding did not cause a physiological drought in these plants. Flooding caused stomata to closure (decline of g_s) thus depressing A_{net} and E under both non saline and saline conditions. The effect of flooding on gas exchange is independent of the plant's water status (Herrera, 2013). Stomata closure under such conditions seems to be the result of a signalling process rather than a general loss of turgor (indicated by the high values of Ψ_w and Ψ_p). In fact, flooding (hypoxia) causes a change in the hormonal balance that causes stomata to close and decrease the passive absorption of water by roots. Furthermore the increase of A/g_s ratio under such conditions suggests a stomatal limitation to photosynthesis (g_s decreased far more than A_{net}). Yet, the effect of flooding on photosynthesis was more evident on Miswak plants growing in saline-dry habitats where A/g_s ratio decreased significantly indicating a non-stomatal limitation to the photosynthesis (reduction of the carboxylation activity). This agrees with previous findings showing that stomata closure would be advantageous to a plant of the coastal areas if sudden floods caused important rises in the concentrations of Na^+ and Cl^- in the plant organs (Shabala and Pottosin, 2014; Flowers *et al.*, 2015). The decrease in g_s and A under saline and saline-flooded conditions may have induced chronic photoinhibition. Besides, flooding interacts with salinity to largely increase $iWUE$. $iWUE$ is habitually used as an indicator of salt tolerance since higher soil salinity causes $iWUE$ to increase in halophytes such as *S. fruticosa*, *B. vulgaris* ssp. *maritima* and *A. portulacoides* (Debez *et al.*, 2008; Redondo-Gómez *et al.*, 2010).

Since no salt glands or bladders were found on the leaves of *S. persica* and no indication in the literature on the presence of such structures, it appears that this plant has a strong ability to control uptake and favour extrusion and compartmentalization of Na^+ if it becomes excessive in the soil. Our results revealed that Na^+ concentration in the leaves of plants growing in the sabkha was almost 20-fold higher than those growing in non-saline sites. The build-up of high concentrations of Na^+ in saline-dry site plants, mostly in the leaves, indicates an effective compartmentation of salt in the vacuoles, a key factor in salt tolerance of most halophytes (Flowers *et al.*, 2015). Salt compartmentation mechanism at organ level was demonstrated in Miswak, where the toxic ions Na^+ and Cl^- accumulate in the old senescing leaves, the lower parts (roots), and bark thus preventing their built up in photosynthetically active tissues (Rao *et al.*, 2004). This allows the plant to grow well even on otherwise excessively saline soils.

Flooding further increased the concentration of Na^+ in the shoots of Miswak plants growing in saline sites. This suggests that flooding stress (hypoxia) affects the selectivity of the cell membranes as well as the transport of nutrients and the retention of ions and small metabolites by roots (Di

Bella *et al.*, 2015). Furthermore, soil salinity reduced K^+ concentration and K^+/Na^+ ratios in Miswak leaves. The decline in K^+ concentration was described in several halophytes (Redondo-Gómez *et al.*, 2010; Flowers *et al.*, 2015) and glycophytes (Tounekti *et al.*, 2012; Ben Rejeb *et al.*, 2015). This is generally attributed to the dislocation of K^+ by Na^+ from soil colloids and absorbing surfaces of roots (Flowers *et al.*, 2015). In fact, Na^+ uptake induces cell membrane depolarization which causes an outward-movement of K^+ (Shabala and Pottosin, 2014). Interestingly, our results indicate that the capacity to transport K^+ over Na^+ from root to stem ($ST_{(root/stem)}$) was maintained high in Miswak plants subjected to high medium salinity. A high $ST_{(root/stem)}$ values indicates a better capacity to transport K^+ over Na^+ from the roots to the stems (Debez *et al.*, 2004). Still the plants preferably transported Na^+ over K^+ from the stem to the leaves when the soil was saline (lower $ST_{(stem/leaf)}$ values). It is likely that these toxic ions will be later excluded in the senescing leaves (Rao *et al.*, 2004). Flooding further increases leaf senescence of salt-affected plants (Maggio *et al.*, 2000; Barrett-Lennard and Shabala, 2013).

Like most halophytes, *S. persica* depends highly on inorganic ions (Na^+ , Cl^- , and K^+) for osmotic adjustment and maintenance of cell turgor. For instance, Na^+ and K^+ contribute around 40% to Ψ_π under saline conditions. The combination of salinity and flooding further increased the contribution of these two cations to reach 69%. Generally, Na^+ , Cl^- , and K^+ contribute 80–95% of plant Ψ_π under salinity conditions for most halophytes (Flowers *et al.*, 2015). The contribution of proline increased to about 5% by salinity. Because this compatible solute accumulates mainly in the cytosol which represents a small fraction of the total volume of a mature cell, its role in osmotic adjustment can still be important (Flowers *et al.*, 2015). Proline also plays a major role in osmo-protection against free radicals (Debez *et al.*, 2008). TSS content decreased considerably in plants growing under stress due to flooding, salinity or both. It appears that TSS accumulated in the roots rather than in the leaves. This is in agreement with previous reports indicating that flood-tolerant plants accumulate large amounts of carbohydrate in their roots (Crawford and Braendle, 1996).

Chl $a+b$ and carotenoid contents decreased slightly due to salinity of the sabkha. Such loss of Chl $a+b$, common in many halophytes (Flowers *et al.*, 2015) and glycophytes (Tounekti *et al.*, 2012; Ben Rejeb *et al.*, 2015), can fulfil a similar protecting function as photoinhibition and the decrease of ETR at acute and prolonged salinity conditions (Koyro *et al.*, 2013). Our results reveal also that flooding further decreased Chl $a+b$ content, but increased slightly carotenoids concentration. The loss of Chl $a+b$ is related to changes in the photosynthetic characteristics of leaves. The plants growing in areas with unstable soil salinity and sporadic flooding, such as *S. persica*, may have various photo-protective mechanisms to deal with either salinity, flooding (hypoxia) or their combination in the medium. For

instance, in *S. persica* plants growing in non-saline habitats, flooding did not increase NPQ nor decrease Fv/Fm in any meaningful way indicating that no photoinhibition of the photosynthesis occurred. Besides, since Φ_{PSII} is an indicator of electron supply to photosynthesis (and photorespiration) (Demmig-Adams *et al.*, 1996), the higher Φ_{PSII} and A_{net} manifested by Miswak plants growing in non-saline-flooded habitats, indicate that adequate electron transport feeds these two processes. Therefore, we suggest that photorespiration and cyclic electron transport are protecting leaf tissues without increasing the thermal dissipation of the PSII antennae as shown previously (Redondo-Gómez *et al.*, 2010). It can be inferred that flooding is not a real stress for *S. persica*. This confirms also the common idea that flooding is far from constituting a stress to plants; to the contrary it is a natural part of the set of ecological variables that make the presence of the species in such ecosystem possible (Herrera, 2013). Even under saline-dry habitats (sabkha) and despite visible signs of physiological stress, no photoinhibition was detected as Fv/Fm values were always higher than 0.7, the threshold proposed previously below which photo-inhibition occurs (Demmig-Adams *et al.*, 1996; Baker, 2008). Consequently, it appears that thermal dissipation of excess energy (increased NPQ) concomitant with the decrease of Φ_{PSII} might have protected PSII from over excitation under such stressful conditions (Naumann *et al.*, 2007). Such photo-protection commonly involves the xanthophyll cycle in addition to adjustments to the composition of light harvesting antennae; it protects proteins, pigments, and lipids of cell membranes from oxidative damage (Tounekti *et al.*, 2012). Furthermore, Fv/Fm ratio of Miswak was not affected by high soil salinity; a similar response has been reported previously in many halophytes (Debez *et al.*, 2008; Redondo-Gómez *et al.*, 2010). Whereas, in the salt-sensitive glycophytes, Fv/Fm decreases noticeably (Ben Rejeb *et al.*, 2015). Under saline-flooded conditions, Fv/Fm decreased to below 0.7 indicating the occurrence of photo-inhibition despite the apparent increase in NPQ. In *S. persica*, ETR, q_p , Φ_{PSII} and A decreased in plants growing either in saline-dry or saline-flooded habitats which suggests a reduction in the flow of electrons through the photosystems in order to prevent photosynthetic reaction centres from being over excited as proposed by Koyro *et al.* (2013). Hence, it appears that salt tolerance of *S. persica* is partly due to its aptitude to preserve the integrity of PSII. Moreover, lipid peroxidation as indicated by MDA content increased only slightly in the plants growing in the sabkha and was not affected by flooding suggesting that Miswak has an efficient antioxidative defence system. Therefore neither the decrease of Chl $a+b$ content nor the increase of heat emission (NPQ) have reduced sufficiently the flow of electrons through photosystems to prevent the photosynthetic reaction centres in the leaves of *S. persica* from over excitation if the area is saline and flooded inducing photo-inhibition of the photosynthetic machinery. Commonly similar conditions induce oxidative stress in both halophytes and glycophytes.

Such decrease was also seen under flooding stress alone where plants did not suffer an intense physiological stress. This confirms that flood tolerance is related to the capacity of roots to accumulate carbohydrate (Crawford and Braendle, 1996).

In conclusion, our results confirm that *S. persica* is a facultative halophyte that can survive contrasting edaphic conditions thanks to efficient adaptation strategies at different levels of organisation, which help it cope with ambient stresses such as salinity, hypoxia and heat. Both SD and g_s were regulated according to stress type in order to adjust leaf gas-exchange. *S. persica* is able to regulate its shoot ion contents despite of the hypoxic medium by controlling uptake, favouring compartmentalization of Na^+ in the vacuoles and its later extrusion with the senescing leaves. Still these ions, in addition to K^+ , contribute significantly to osmotic adjustment in leaf tissues. Proline accumulation in the leaves and roots seems to play a role in osmotic adjustment and osmo-protection. Our results indicate that TSS does not appear to play an important role in coping with stress. Furthermore, various photo-protection mechanisms appear to help the plant overcome the consequences of these abiotic stresses. A combination of morphological (smaller leaves) and physiological (accumulation of Na and K in the leaves) adjustments and increased osmo- (accumulation of proline) and photo- (reduction in chlorophyll content, increased non-photochemical quenching and reduced Photochemical quenching (q_p) and linear electron transport rate) protection helped the plant overcome salinity, hypoxia and their combination in its native habitats.

Conclusion

Consequently, due to its ability overcome multiple and simultaneous stresses, *S. persica* can be considered an excellent woody perennial species to replant degraded sabkhas and coastal habitats. In addition to its role in folk medicine and as a potential source of bioactive compounds, the plant is also a good source of fodder for livestock and an excellent habitat for wildlife.

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References

- Ahmad, H. and K. Rajagopal, 2013. Biological Activities of *Salvadora persica* L. (Meswak). *Med. Aromat. Plants*, 2: 129
- Al-Jaloud, A.A., M.Y. Al-Saiady, A.M. Assaeed, and S.A. Chaudhry, 2001. Some halophyte plants of Saudi Arabia, their composition and relation to soil properties. *Pak. J. Biol. Sci.*, 4: 531–534
- Amon, D.I., 1949. Copper enzyme in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. *Plant Physiol.*, 24: 1–15

- Baker, N.R., 2008. Chlorophyll fluorescence: a probe of photosynthesis in vivo. *Annu. Rev. Plant Biol.*, 59: 89–113
- Barrett-Lennard, E.G. and S.N. Shabala, 2013. The waterlogging/salinity interaction in higher plants revisited – focusing on the hypoxia-induced disturbance to K⁺ homeostasis. *Funct. Plant Biol.*, 40: 872–882
- Bates, L.S., R.P. Waldren and I.D. Teare, 1973. Rapid determination of free proline for water-stress studies. *Plant Physiol.*, 39: 205–207
- Ben Rejeb, K., M. Benzartia, A. Debez, C. Bailly, A. Savouré and C. Abdelly, 2015. NADPH oxidase-derived H₂O₂ production is required for salt-induced antioxidant defense in *Arabidopsis thaliana*. *J. Plant Physiol.*, 174: 5–15
- Black, C.A., 1965. *Methods of Soil Analysis: Part I Physical and mineralogical properties*. American Society of Agronomy, Madison, Wisconsin, USA
- Colmer, T.D. and L.A.J.C. Voesenek, 2009. Flooding tolerance: suites of plant traits in variable environments. *Funct. Plant Biol.*, 36: 665–681
- Crawford, R.M.M. and R. Braendle, 1996. Oxygen deprivation stress in a changing environment. *J. Exp. Bot.*, 47: 145–159
- Dabbagh, A.E. and W.A. Abderrahman, 1997. Management of groundwater resources under various irrigation water use scenarios in Saudi Arabia. *Arab. J. Sci. Eng.*, 22: 47–64
- Debez, A., K. Ben Hamed, C. Grignon and C. Abdelly, 2004. Salinity effects on germination, growth, and seed production of the halophyte *Cakile maritime*. *Plant Soil*, 262: 179–189
- Debez, A., H.W. Koyro, C. Grignon, C. Abdelly and B. Huchzermeyer, 2008. Relationship between the photosynthetic activity and the performance of *Cakile maritima* after long-term salt treatment. *Physiol. Plant.*, 133: 373–385
- Demmig-Adams, B., W.W. Adams, D.H. Baker, B.A. Logan, D.R. Bowling and A.S. Verhoeven, 1996. Using chlorophyll fluorescence to assess the fraction of absorbed light allocated to thermal dissipation of excess excitation. *Physiol. Plant.*, 98: 253–264
- Di Bella, C.E., A.A. Grimoldi, M.S. Rossi Lopardo, F.J. Escaray, E.L. Ploschuk and G.G. Striker, 2015. Differential growth of *Spartina densiflora* populations under saline flooding is related to adventitious root formation and innate root ion regulation. *Funct. Plant Biol.*, 43: 52–61
- El-Demerdash, M.A., A.K. Hegazy and A.M. Zilay, 1995. Vegetation-soil relationships in Tihamah coastal plains of Jazan region, Saudi Arabia. *J. Arid Environ.*, 30: 161–174
- Flowers, T.J., R. Munns and T.D. Colmer, 2015. Sodium chloride toxicity and the cellular basis of salt tolerance in halophytes. *Ann. Bot.*, 115: 419–431
- Ge, Z.M., H. Wang, H.B. Cao, B. Zhao, X. Zhou, H. Peltola, L.F. Cui, X.Z. Li and L.Q. Zhang, 2016. Responses of eastern Chinese coastal salt marshes to sea-level rise combined with vegetative and sedimentary processes. *Sci. Rep.*, 6: 28466
- Gorai, M., M. Ennajeh, H. Khemira and M. Nefati, 2010. Combined effect of NaCl-salinity and hypoxia on growth, photosynthesis, water relations and solute accumulation in *Phragmites australis* plants. *Flora*, 205: 462–470
- Herrera, A., 2013. Responses to flooding of plant water relations and leaf gas exchange in tropical tolerant trees of a black-water wetland. *Front Plant Sci.*, 4: 106
- Hodges, D.M., J.M. DeLong, C.F. Forney and R.K. Prange, 1999. Improving the thiobarbituric acid-reactive-substances assay for estimating lipid peroxidation in plant tissues containing anthocyanin and other interfering compounds. *Planta*, 207: 604–611
- Koyro, H.W., T. Hussain, B. Huchzermeyer and M.A. Khan, 2013. Photosynthetic and growth responses of halophyte grass *Panicum turgidum* to increase NaCl concentrations. *Environ. Exp. Bot.*, 91: 22–29
- Maggio, A., M.P. Reddy and R.J. Jolly, 2000. Leaf gas exchange and solute accumulation in the halophyte *Salvadora persica* grown under moderate salinity. *Environ. Exp. Bot.*, 44: 31–38
- Naumann, J.C., D.R. Young and J.E. Anderson, 2007. Linking leaf optical properties to physiological responses for stress detection in coastal plant species. *Physiol. Plant.*, 13: 422–433
- Osnas, J.L., J.W. Lichtstein, P.B. Reich and S.W. Pacala, 2013. Global leaf trait relationships: mass, area, and the leaf economics spectrum. *Science*, 340: 741–744
- Poorter, H. and E. Garnier, 2007. Ecological significance of inherent variation in relative growth rate and its components. In: *Functional plant ecology*, pp: 67–100. Pugnaire, F.I. and F. Valladares (eds.). CRC Press, New York, USA
- Rangani, J., A.K. Parida, A. Panda and A. Kumari, 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
- Rao, G.G., A.K. Nayak, A.R. Chinchmalapur, A. Nath and V.R. Babu, 2004. Growth and yield of *Salvadora persica*, a facultative halophyte grown on saline black soil (Vertic Heplustept). *Arid Land Res. Manage.*, 18: 51–61
- Reddy, M.P., M.T. Shah and J.S. Patolia, 2008. *Salvadora persica*, a potential species for industrial oil production in semiarid saline and alkali soils. *Ind. Crops Prod.*, 28: 273–278
- Redondo-Gómez, S., E. Mateos-Naranjo, M.E. Figueroa, and A.J. Davy, 2010. Salt stimulation of growth and photosynthesis in an extreme halophyte, *Arthrocnemum macrostachyum*. *Plant Biol.*, 12: 79–87
- Richards, L.A., 1954. *Diagnosis and Improvement of Saline-alkali Soils*. Agricultural Handbook USDA 60. USDA, Washington DC, USA
- Roças, G. and F.R. Scarano, 2001. Leaf anatomical variation in *Alchornea triplinervia* (Spreng) Müll. Arg. (Euphorbiaceae) under distinct light and soil water regimes. *Trop. Ecol.*, 47: 243–250
- SAS Institute, 1996. *SAS Institute User's Guide: Statistics, Version 6*. Cary, North Carolina, USA
- Shabala, S. and I. Pottosin, 2014. Regulation of potassium transport in plants under hostile conditions: implications for abiotic and biotic stress tolerance. *Physiol. Plant.*, 151: 257–279
- Li, S.H., Z.M. Ge, L.N. Xie, W. Chen, L. Yuan, D.Q. Wang, X.Z. Li and L.Q. Zhang, 2018. Ecophysiological response of native and exotic salt marsh vegetation to waterlogging and salinity: Implications for the effects of sea-level rise. *Sci. Rep.*, 18: 2441
- Tounekti, T., M.E. Abreu, H. Khemira and S. Munné-Bosch, 2012. Canopy position determines the photoprotective demand and antioxidant protection of leaves in salt-stressed *Salvia officinalis* L. plants. *Environ. Exp. Bot.*, 78: 146–156
- Voss, C.M., R.R. Christian and J.T. Morris, 2013. Marsh macrophyte responses to inundation anticipate impacts of sea-level rise and indicate ongoing drowning of North Carolina marshes. *Mar. Biol.*, 160: 181–194
- WHO, 1987. Preventive Methods and Programmes for Oral Diseases
- Wright, I.J., P.B. Reich, M. Westoby, D.D. Ackerly, Z. Baruch, F. Bongers, J. Cavender-Bares, T. Chapin, J.H.C. Cornelissen, M. Diemer, J. Flexas, E. Garnier, P.K. Groom, J. Gulias, K. Hikosaka, B.B. Lamont, T. Lee, W. Lee, C. Lusk, J.J. Midgley, M.L. Navas, U. Niinemets, J. Oleksyn, N. Osada, H. Poorter, P. Poot, L. Prior, V.I. Pyankov, C. Roumet, S.C. Thomas, M.G. Tjoelker, E.J. Veneklaas and R. Villar, 2004. The worldwide leaf economics spectrum. *Nature*, 428: 821–827
- Yemm, E.W. and A.J. Willis, 1954. The estimation of carbohydrates in plant extracts by anthrone. *Biochem. J.*, 5: 508–514
- Zodape, S.T. and V.K. Indusekhar, 1997. *Salvadora persica*: a boon to wasteland development. *J. Sci. Ind. Res.*, 56: 657–661

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