Leaf Functional Trait Variation Associated with Salinity Tolerance in \textit{Salvadora persica}

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ABSTRACT

Morphological leaf traits that mediate environmental effects on plant fitness are often regarded as ‘functional traits’. These traits influence plant physiological responses to their immediate environment, which in turn affect performance, growth and survival. The objective of this study was to determine the effects of the medium salinity on several plant traits including functional leaf traits, gas exchange, and leaf stomatal density of \textit{Salvadora persica}. We considered how functional leaf traits such as leaf mass area (LMA), specific leaf area (SLA), succulence index (S), leaf dry mass contents (LDMC) and leaf thickness (Lth) differs between populations growing in nonsaline (ECe 5 dS m$^{-1}$) and saline habitats (ECe 25 dS m$^{-1}$). Our results showed a significant decrease (27%) in leaf area (L$_{A}$) of \textit{S. persica} growing under saline habitats. The leaf characteristics reveal that this facultative halophyte adapted to increased salinity by shifting from fast-growth to slow-growth with conservation of water and nutrients. For instance, the SLA decreased while LMA increased for plants growing under saline condition. The lower values of SLA (or higher values of LMA) contribute to lengthening leaf life-span, nutrient retention, and protection from dehydration of plants. However, the L$_{th}$, LDMC and S increased under such conditions. The increases in LDMC correlated with decreases in the photosynthetic assimilation (A). In fact, our results showed that the medium salinity induced both stomatal closure (decline of g$_{s}$) , lower leaf stomata density (SD) and reduced A. Consequently, A/g$_{s}$ ratio decreased slightly indicating a non-stomatal limitation to the photosynthesis. Besides, the medium salinity has increased the instantaneous water use efficiency (iWUE), which is often considered as an indicator of salt tolerance.

Key words: Saline habitats, specific leaf area, leaf thickness, water use efficiency, stomata density

INTRODUCTION

Soil salinity is considered as being responsible for the reduction in plant growth and yield in several arid and semi-arid regions of the world. Salt stress reduces shoot growth and leaf expansion through both osmotic effects that induce water deficit and specific ion effects that may cause toxicity and mineral deficiencies. 

The mechanisms of salt tolerance are both complex and diverse, particularly in plants growing naturally on saline soils (halophytes) which have developed several mechanisms to cope with salinity. In general, the tolerance of all halophytes to ionic as well as osmotic stress depends on controlled uptake, improved extrusion and compartmentalization of toxic ions. However, these mechanisms are complex and depend upon anatomical and physiological changes happening in the whole-plant rather than a single cell. At the whole-plant level, acclimation to salt stress is considered an integrated response of different organs, mainly roots and leaves. Although the roots constitute the primary barrier to salt uptake, leaves have received more attention from researchers, because they are the sites of carbon assimilation and, then, more directly allied to plant growth and development. Still, the importance of leaf physiology and morphology on whole-plant salt tolerance was not well understood. Hence, the objective of this paper was to determine the effects of the soil salinity on morpho-physiological changes in Salvadora persica leaves. Morphological leaf traits that mediate environmental effects on plant fitness are often regarded as ‘functional traits’. These often easy-to-measure traits influence plant physiological responses to their immediate environment, which in turn affect performance, growth and survival.

Salvadora persica, popularly called meswak, is an evergreen shrub or small tree (6-7 m high) native of subtropical to tropical Africa. The main trunk is erect or trailing and profusely branched. Large brushes of S. persica are prominent in many parts of the Southwestern Saudi Arabia. This facultative halophyte is adapted to survive various adverse environmental conditions ranging from nonsaline to highly saline soil (EC more than 30 dS m⁻¹), and dry regions to marshy and waterlogged areas. 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 some coastal plains, S. persica is located at downstream (deltas) of major Wadis. Meswak is also prevalent in the beds of wadis and near lakes. This variability in its habitat may explain the great genetic variability within and between the several populations encountered.

Studies on the biology, physiology and medicinal usage of meswak were previously reviewed. It was shown that S. persica species possesses a high potential economic value as a source of oil and medicinal compounds. The plant contains several bioactive compounds like alkaloids, tannins, saponins potentially interesting for the food and cosmetic industries. Its roots are used as tooth brush and constitute a good source of some pharmaceutical derivatives. The foliage is used as source of lipids, gum and resins. Since it grows both inland and in coastal wetland, S. persica has high ecological value in arid and semiarid ecosystems. It is recommended for the reclamation of both sand dunes and saline soils habitats. However, the present stands in Tahama plains of southwestern Saudi Arabia are under great pressure for over-grazing and uprooting. Hence the need to protect certain areas, where this species, is well established 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. This study evaluated the effects of salinity on some plant traits including functional leaf traits, photosynthesis and leaf stomatal density to better appreciate the response of meswak to salinity. We considered how functional leaf traits such as leaf mass area (LMA), specific leaf area (SLA), succulence index (S), and leaf thickness (Lth) differs between habitats (populations). In fact, these leaf traits are considered as fundamental for ecosystem functioning, being related with vital processes such as carbon gain and litter decomposability.
MATERIAL AND METHODS

The study area and plant material

The study area is located in Jazan region in the southwestern Saudi Arabia near the Saudi/Yemeni border. The area has a subtropical desert climate, where the average annual temperature is 23°C; the average relative humidity varies between 45% and 65% in winter and 25% and 40% in summer. The annual average rainfall varies from 100 to 250 mm in the coastal plain area and 500 to 700 mm in the eastern mountains.

S. persica species from different populations (localities) were used in this study. The halophytic community of S. persica dominates the holomorphic and hydromorphic soils of the sabkhas. 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. A number of stands of meswak were selected for sampling in two different habitats of S. persica: Two sites were selected at the area west of Abu-Arish (non-saline habitat), and two sites representing the saline habitat of the coastal area (sabkhas) in the north of Jazan city. At each site, eight bushes of S. persica were tagged for sampling. Healthy-looking, fully expanded leaves were collected and transported to the laboratory.

Soil salinity measurement

Soil samples (approx. 5 kg) were taken at a depth of 20 cm from the area adjacent to the tree roots, and placed directly in plastic bags to avoid moisture loss. Part of each samples was air dried and sieved to pass through a 2 mm mesh and its electrical conductivity (ECe in dS m⁻¹) was determined in soil–water extracts at 1:1 (w:v).

Functional leaf traits analysis and plant water relations

The specific leaf area (SLA), leaf mass area (LMA), leaf succulence (S), leaf dry matter content (LDMC), and leaf water content (LWC) were determined on 10 leaves per plant (8 plants from each of the four sites). Leaf areas (Lₐ) were estimated using a photo scanner (Epson Perfection 4870; Epson America, Inc., Long Beach, CA, USA) and analysed by the ImageJ software. The LMA was calculated as the ratio of LDMC/Lₐ, SLA was calculated as SLA = Lₐ/LₐFW. The components leaf density (L₊), and leaf thickness (Lₜh) increase linearly with the inverse of SLA: 1/SLA = L₊×Lₜh. Leaf density was estimated by calculating LDMC because L₊ ≈ LDMC, and LDMC = LFW/L₁FW 14, with L₁FW as leaf dry weight (g) and LFW as leaf fresh weight (g). L₁FW is the dry weight after oven-drying the leaves at 80°C for 48 h. Leaf thickness (Lₜh) was determined as: Lₜh = (SLA×LDMC)⁻¹. This Lₜh can also be stated as leaf succulence because (SLA×LDMC)⁻¹ = (L₊×L₁FW/L₁FW)⁻¹ = LFW/L₁A, and LFW/L₁A is often used as an estimate of leaf succulence. Leaf succulence (S) is calculated as flow S = LFW/L₁A, with LFW as leaf fresh weight (g) and L₁A as leaf area (cm²). Leaf water content was calculated according to the following formula: LWC = (L₁FW−L₁DW)/LFW.

Gas exchange measurements

Photosynthetic assimilation (A) and stomatal conductance (gs) were measured using a CIRAS-2- Portable Photosynthesis System (PP system, USA). The measurements were carried out on fully expanded healthy-looking leaves between 9 a.m. and 11 a.m. at incident PPFD of 1500 to 2100 μmol m⁻² s⁻¹ and with an air temperature ranging from 29°C to 33°C. Instantaneous water use efficiency (iWUE) was calculated by the following formula: iWUE = A/gs. Intrinsic water use efficiency was also calculated as A/gs ratio.

Stomatal densities

To determine stomatal (SD) on the abaxial and adaxial leaf-surfaces, a thin layer of nail polish was applied to the epidermis of the leaf after trichomes had first been removed using adhesive tape. Once dry, the nail polish layer was carefully peeled-off with adhesive tape, then fixed on a microscope slide and examined under a light microscope (MCX300; Micros, Vienna, Austria) equipped with a camera interfaced to a computer. The numbers of stomata scars per unit leaf area were then determined. Five leaves per pant were used.
Statistical analyses

The significance of differences between measurements for all dependent variables was analysed using the GLM procedure (ANOVA test) of SAS software\(^1\) with sixteen replicates for each habitat (eight replicates from each of two sites). Each tree was considered as a replicate. Differences were considered significant when \(P \leq 0.05\) (probability level).

RESULTS

Soil salinity

Our results showed that the salinity of the sabkha soil varies from site to site and from season to season. The soil ECe ranges between 19.5 and 25.7 dSm\(^{-1}\). The texture of sabkhas soils ranges from coarse (sandy) to fine (sandy-clay-loam) depending on location.

Functional leaf traits

Our results demonstrate that the salinity of the medium (ECe between 19.5 and 25.7 dS m\(^{-1}\)) decreased by 27% the leaf area (L\(_A\)) of \(S.\) persica (Figure 1). The specific leaf area (SLA), leaf mass area (LMA), leaf dry matter content (LDMC), leaf thickness (L\(_th\)) and succulence index (S) have been used in this study as indicators on plant growth, leaf venture strategies, and later on the plant functioning. The present study showed that SLA decreased while LMA and LDMC (leaf density) increased for \(S.\ persica\) growing in saline (sabkha) habitats (Figure 1). The leaf thickness (L\(_th\)) and LDMC (leaf density, L\(_D\)) increased under saline habitats. The leaf succulence index (S) increased significantly under the effect of the soil salinity.

Plant water content and gas exchange

Our results revealed that salinity affected considerably the leaf water content (LWC). Furthermore, both \(g_s\) and \(A\) decreased considerably under such conditions (Figure 2). Still the decrease of the \(g_s\) was more acute than the decrease of \(A\). In contrast, iWUE (A/E) increased slightly as expected.

Stomata density

Our results showed that the stomata density (SD) was significantly higher in the adaxial surface of the leaves compared to the abaxial surface in both habitats (Figure 3). The salinity of the medium decreased the stomatal density (SD) on both the abaxial and adaxial surfaces.

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**Fig. 1:** Leaf area, succulence index, leaf mass area (LMA), specific leaf area (SLA), Leaf dry mass content (LDMC), and leaf succulence (S) of the \(S.\ persica\) leaves collected from populations growing under non saline and saline conditions. The results are mean of at least eight replicates ± SE; different letters indicate significant differences at \(P < 0.05\) in response to the plant habitat (Duncan test).
Fig. 2: Leaf water content (LWC), stomatal conductance (gs), photosynthetic assimilation (A) and instantaneous water use efficiency (iWUE) of *S. persica* plants living in diverse habitats in Jazan region of Saudi Arabia. The results are mean of at least eight replicates ± SE; different letters indicate significant differences at *P* < 0.05 in response to the plant habitat (Duncan test).

Fig. 3: Stomatal density on adaxial and abaxial surfaces of the leaves of *S. persica* plants living in diverse habitats in Jazan region of Saudi Arabia. The results are mean of at least eight replicates ± SE; different letters indicate significant differences at *P* < 0.05 in response to the plant habitat (Duncan test).

**DISCUSSION**

Soil salinity is responsible for the reduction in plant growth and yield in arid and semi-arid regions of the world. The decline in leaf expansion is one of the earliest responses of plants to several abiotic stresses. The present results showed a significant decrease (27%) in individual leaf area (Lₐ) of *S. persica* growing under saline conditions (ECₑ between 19.5 and 25.7 dS m⁻¹). The reduction in Lₐ caused by the inhibition of leaf expansion may be considered as an adaptive mechanism that...
allows plants to survive elevated soil salinity for a longer period of time. The inhibition of the leaf expansion by salinity has been observed in many other plant species. The process of leaf growth inhibition can be allied with osmotic and toxic effects of salt stress. Previous reports showed that under salt stress conditions, the cells are smaller, with thicker walls and they are more tightly packed, with a lower fraction of air spaces. These adaptations of leaf tissues increase leaf density. Such decrease in leaf expansion can save energy, minimize toxic reactive oxygen species (ROS) production, reduce amino acid demand for protein synthesis, and thus offer more free amino acids for osmotic adjustment. Furthermore, the decrease of Lmin limits water loss by transpiration and thus controls the transport of toxic ions to the shoots by enhancing their allocation in roots.

It was stated that different ecological conditions may impose different selective pressures on plants, driving traits to a certain degree of divergence. In this regard, leaf traits are fundamental for ecosystem functioning, being related with vital processes such as carbon gain or litter decomposability. The examination of several functional leaf traits reveals that S. persica adapted well to the salinity of the medium. The SLA, LMA, LDMC, Lth and S have been used in this study as indicators of plant growth and leaf adaptation strategies. SLA reveals resource-uptake efficiency, by increasing the assimilation area per unit of biomass. SLA is highly correlated with net photosynthetic rate (A) and plant growth rate. Like in many other halophytes, SLA decreased (or LMA increased) while LDMC (or Lth) increased for miswak plants growing under saline condition (sabkha). The decrease in SLA with increasing salinity has been reported for Lycopersicon esculentum and for Aster tripolium. Still halophytes, as meswak, are better than glycophytes in excluding salts, which may be the reason that halophytes decrease SLA (increase LMA) only at higher salinities and relatively less strongly than glycophytes. The lower values of SLA (or higher values of LMA) contribute to long leaf life-span, nutrient retention, and protection from dehydration of plants. Also it indicates that more dry matter is allocated to each leaf because nutrients are less available in saline soils. It was reported that LMA (or SLA) is highly correlated with leaf processes such as maximum photosynthetic rate, whole-plant activities such as the species’ potential growth rate and ecosystem processes such as litter decomposition rate.

As for many halophytes, the Lth and LDMC (Lth) of S. persica increased under saline conditions. Leaf thickness (Lth) plays an important role in leaf and plant functioning and is related to species’ strategies of resource acquisition and use. The amount of light absorbed by a leaf, and the diffusion pathway of CO2 through its tissues depend, at least partially, on its thickness. Negative relations between Lth and photosynthetic and growth rates have been observed, and thicker leaves have sometimes been associated with increased longevity and construction costs. Lth has so often been used as a tool to screen species and/or cultivars for productivity or ecological performance. Furthermore the succulence index (S) increased under the medium salinity. Such response of S. persica is considered an adaptation in order to save water and dilute toxic ions in halophytes. Since the photosynthetic carbon acquisition by a leaf depends not only on Lmin, but also on Lth, an increase in succulence can compensate for the negative effects of salinity on leaf cell metabolism to some extent.

Generally, the anatomical changes which occur in response to stress are designed to optimize leaf gas-exchange. In fact, our results show that meswak plants grown in saline habitats had lower stomata density (SD). This might be interpreted as a key adaptive feature designed to reduce leaf transpiration. In relation to this, salinity of the medium induced both stomatal closure (decline of gs) and reduced the photosynthetic assimilation (A). A/gs ratio decreased too slightly indicating a non-stomatal limitation to the photosynthesis (reduction of the carboxylation activity).
agrees with previous findings showing that stomata closure would be advantageous to a plant in saline habitats. Besides, salinity has increased the iWUE. Such parameter is often considered as an indicator of salt resistance as higher soil salinity cause iWUE to increase in halophytes such as *S. fruticosa*, *B. vulgaris* ssp. maritima and *A. portulacoides*. In conclusion, our results confirm that *S. persica* is a facultative halophyte that can survive contrasting ecological systems and displays efficient adaptation strategies to cope with ambient stresses due to salinity. Functional leaf traits reveal that this species overcomes stresses by shifting from fast-growth to slow growth in order to save water and nutrients. Both SD and gs were regulated according to the plant habitat (either saline or not) in order to adjust leaf gas-exchange.

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REFERENCES


