



American Journal of
Plant Physiology

ISSN 1557-4539



Academic
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Research Article

Autecology and Physiological Features of *Salvadora persica* Plants Grown under Dry Conditions

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Abstract

Background and Objectives: *Salvadora persica* L. is specie of halophytes occurs in arid and semi-arid regions of Asia and Africa. The aims of the study was to optimize the physiological state of *S. persica* and its stability in dry environment **Materials and Method:** *Salvadora persica* L., plant had been collected from Rabigh province and been subjected to physiological state evaluation. The evaluation included leaf area, photosynthetic parameters and transpiration rate, trehalose content and catalase activity. The leaf area was 4.71 cm². **Results:** The maximum net rates of photosynthesis (A) and transpiration (E) were 53.96 and 6.44 mmol m⁻² sec⁻¹, respectively. The photosynthetic parameter of *S. persica* leaf were, Vapor pressure deficit, stomatal conductance to water vapor, substomatal CO₂, water use efficiency and photosynthetic active radiance has been measured. In *S. persica* L., shoot, the trehalose content was 4.33 µg g⁻¹ dry weight and catalase activity (CAT) was 3.58 mM H₂O₂ g⁻¹ fresh weight min⁻¹. Chemical ions have been analyzed both in plant shoots and the soil rhizosphere. The remarkable points of these analyses are the presence of high level of Ca²⁺ in plant shoots and high level of Fe²⁺ in the soil rhizosphere. **Conclusion:** This study established that *S. persica* grew and developed well in this dry area. As well as, it can be used to fix sands, indeeds the fixation represents a point of interest for biologists and economists.

Key words: *Salvadora persica*, autecology, photosynthetic parameters, transpiration rate, antioxidant enzymes, trehalose

Citation: Ghalia S. Aljeddani and Hanaa E. Ahmed, 2020. Autecology and physiological features of *Salvadora persica* plants grown under dry conditions. Am. J. Plant Physiol., 15: 14-22.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Autecology is a branch of ecology deals with biological relationship between individual organism or species and its surrounding environment. Rabigh Province is of great interest from an ecological and biological point of view, especially because of its geographic and climatic changes. It is characterized by a desert climate. During the year, there is virtually no rainfall. The climate in Rabigh area is classified as BWh (hot desert)^{1,2}. The average annual temperature is 28.6°C in Rabigh. July is the hottest month of the year (33.7°C). However, January is the coldest month of the year (22.2°C). Precipitation average is 44 mm. The lowest precipitation is in February, with an average of 0 mm. The most precipitation falls in November, with an average of 16 mm (<https://en.climate-data.org/asia/saudi-arabia/makkah-region/rabigh-53335/>).

Salvadora persica (Arak or Miswak tree) belongs to family Salvadoraceae³. It is an evergreen perennial halophyte capable of growing under extreme conditions of dry environments and saline soils⁴. It has numerous deciduous fibrous branches. Leaves are opposite, elliptic-lanceolate or ovate, often mucronate at the apex. The trees possess numerous greenish-yellow flowers with a very thin corolla. The fruit is drupe with 3 mm diameter, it turned to red when ripe⁵.

Salvadora persica has been used in traditional medicine for a number of illnesses. It is a popular tooth brush and is one of the most popular medicinal plants throughout the area of its distribution⁶. Besides its medicinal potentialities, it is also suitable in agroforestry systems as a wind break and helps in land reclamation⁷. The roots are used as Miswak for brushing teeth. Roots have an antibiotic effect and contain many beneficial chemicals. The sand may cover an area for various depth, rather evenly over large tracts forming the sand sheet. Recently *S. persica* obtains more attention as it has environmental impact in combating the desertification, sand encroachment and sand dunes⁸. Sand dunes are considered one of the most obstacles that face the horizontal or vertical expansion of agriculture in the desert.

Salvadora persica grows wildly at a desert climate. Therefore it should have special physiological features that help plant in perennation at this difficult environment. Several groups of quantitative or qualitative parameters exist which have been applied to characterize the plant development and growth for example, photosynthesis and transpiration rates, stomatal conductance, enzyme activities, level of osmolytes compounds such as trehalose etc. Osmolytes which are low-molecular weight organic compounds stabilize the properties of biological fluids⁹. The essential function of the osmolytes is to stabilize the viscosity, melting point and ionic

strength of the aqueous solution of the cell fluids, as well as they influence protein folding¹⁰. Common osmolytes include amino acids, sugars and polyols, methylamines, methyl sulfonium compounds and urea. Measurement of enzyme activities, e.g., superoxide dismutase, catalase (CAT), ascorbate peroxidase or characterization of oxidative damage or lipid peroxidation are also sensitive tools on the biochemical level to characterize the physiological features of the plant¹¹. The record of both macro and micro elements in soil and plant, as well as the EC measurement represent excellent methods for stress indication. The aim of this investigation was to study the physiological state of *S. persica* and prove its ability to grow and develop in the dry environment.

MATERIALS AND METHODS

Study area: The study area of *S. persica* community is located near Rabigh Province, its location Coordinates Latitude: N22°32'44.255" Longitude: E39°08'641". It is an open sandy area surrounded by mountains from all sides and spread by *S. persica* plants. Plant samples and soil were collected for 2 seasons in March-April during 2016 and 2017 (Fig. 1).

Field analysis for photosynthesis and transpiration rates:

Portable photosynthesis system (CIRS-3) had been used to automatically determine relative humidity, plant leaf area, leaf temperature, vapor pressure deficit (VPD), stomatal conductance to water vapor (g_s), substomatal CO₂ (C_i), net photosynthesis (A), transpiration rates (E) water use efficiency (WUE) and photosynthetic active radiance (PAR) in the field of the study area in March- April during 2016 and 2017.

Sampling of plant and soil: Samples were collected during March and April 2016 and 2017. Soil samples were collected from (20-30) cm under the soil plane. Leaves samples were collected randomly from five plants from the study area.

Soil analysis: The 2 mm a plastic sieve was used for the air-dried soil samples for removing large gravel-sized materials. Soil samples were subjected to different investigations, including, pH, EC and some mineral. Electrical conductivity (EC) in a 1:5 soil water extract was estimated according to Rhoades¹². Soil moisture was measured by taking samples from the soil surface of 0-10 cm and depth of 20-30 cm and was dried in the oven at 100°C for two days. The relative soil humidity is calculated as a percentage by the following equation:

$$\text{Soil humidity (\%)} = \frac{\text{Soil weight before oven drying} - \text{Soil weight after oven drying}}{\text{Soil weight after oven drying}} \times 100$$

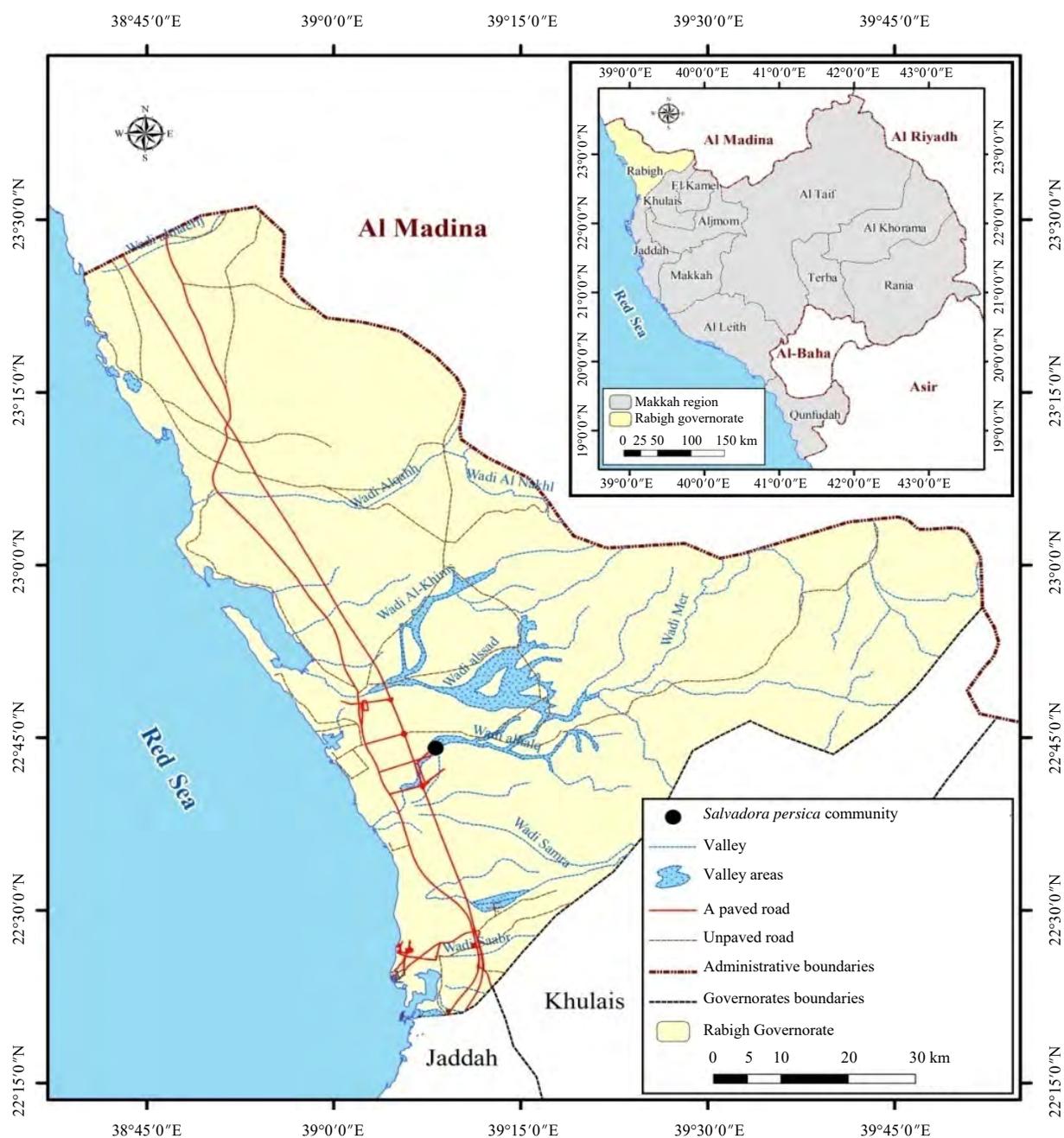


Fig. 1: Location of study area in Rabigh province

Source: Ministry of Petroleum and Mineral Resources, Air Survey Department, Riyadh, Maps Scale 1:250,000, Mecca and Medina Plate, 1410AH, Google Earth 2018

Trehalose determination In *Salvadora persica* shoot: The carbohydrate analysis by GC-MS was carried out according to El-Bashiti *et al.*¹³. Plant samples were harvested at the time mentioned above and ground to a fine powder in liquid nitrogen with a prechilled mortar and pestle. One gram of plant powdered material was transferred to tubes containing

10 $\mu\text{g mL}^{-1}$ phenyl β -galactoside and was placed in water bath at 80°C for 10 min. Insoluble material was removed by centrifugation at 12,000 \times g for 10 min in centrifuge. The supernatants were collected in fresh tubes and the pellets were washed three times in 80% ethanol and centrifuged as before and each wash and the supernatants were pooled with

the first supernatant. The extracts were then concentrated to a volume of 0.5 mL, using a rotary evaporator, transferred to crimp-top vials and dried to a residue at 60°C in an oven. The operating conditions were as follow: Injector 100°C, detector 290°C, oven temperature 100 °C for 3 min. Helium as a carrier gas was used. Ten milligram Trehalose (Sigma) has been used as a standard to determine the quantity of trehalose in the shoot of *S. persica*.

Catalase activity in *Salvadora persica* shoot: Five grams of fresh leaves was homogenized to a fine powder in a pre-chilled mortar by aid of acid washed sand with 15 mL of 50 mM phosphate buffer, pH 7.0 containing 20% (v/v) glycerol, 1 mM EDTA, 5 mM MgCl₂ and 1 mM dithiothreitol (DTT). The extracts were centrifuged at 12000 g for 15 min at 4°C. Extracts were kept at -20°C until determination of enzymatic activities. The CAT (EC 1.11.1.6) activity was measured at 25°C according to Gong *et al.*¹⁴. The CAT activity was estimated by measuring the decrease in absorbance of H₂O₂ at 240 nm. One unit of catalase activity was defined as the amount of enzyme required to oxidize 1 μmol of H₂O₂ min⁻¹ (extinction coefficient 22.4 mM⁻¹cm⁻¹).

Total nitrogen determination in plant sample: The total nitrogen content of the 10 g of dry plant sample was determined using the regular Kjeldahl method¹⁵.

Chemical ions determination: Mineral ions PO₄³⁻, K⁺, Na⁺, Ca²⁺, Mg²⁺, Zn²⁺, Fe²⁺, Si²⁺, MoO₄²⁻, Cl⁻ and carbonate were measured in plant and soil samples during the summer and winter seasons and expressed as mg g⁻¹ dry weight. Plant and soil samples were digested with a mixture of 69% HNO₃ and 30% H₂O₂ (5:2 v/v)¹⁶. The concentrations of chemical ions and metal ions in the digested solutions were determined using inductively coupled plasma-optical emission spectroscopy (Polyscan 61E, Thermo Jarrell-Ash Corp., Franklin, MA, USA).

Statistical analysis: Results are the mean of five samples measurements for each analysis. All obtained data was subjected to ANOVA using SPSS software var. 12. Differences at p ≤ 0.05 were significant.

RESULTS AND DISCUSSION

Rabigh Province is of great interest from an ecological and biological point of view, because of its geographic climatic changes. *Salvadora persica* is a specie of halophytes occurred in the arid and semi-arid regions of the Middle East, Africa and Asia¹⁷. The biotic factors determine the shape

and distribution of *S. persica* and its decline is linked with harvesting intensity, grazing and habitat loss¹⁸.

The study area had been determined using GPS: 2.00 m software ver. 7.50 and represented in Fig. 1, this area is extended between 150 km. The black dot in Fig. 1 indicates the *S. persica* community in Rabigh Province. Photos for vegetation distribution of *S. persica* L. at the study area represented in Fig. 2. In this photo, it was noticed that green bushes of the plant distributed horizontality and on the hill of the elevated land as the land surface is uneven. Flowers and fruits were also observed although the a biotic stressed environments.

To evaluate the physiological condition of the *S. persica* plant and its ability to grow in this area in spite of the dry condition, field analyses have been carried out for leaf area, photosynthetic parameters and transpiration rate on *S. persica* plants and then represented in Table 1. The photosynthetic parameters includes VPD, g_s, C_i, A, E, WUE and PAR. As well as the trehalose content (one of the osmoprotector sugars) and CAT activity (one of antioxidant enzymes).

In this study, *S. persica* leaf area was 4.71 cm². Leaves areas, shape showed great variation in different environments. Investigating relationships between metabolic features which involved CO₂ fixation, water loss by metabolic transpiration affect on plant productivity and adaptation.

The photosynthetic parameters have two values minimum (min) and maximum (max). The minimum value was recorded on old foliage leaf, while the maximum value was recorded on new foliage leaf of *S. persica* (Table 1). This data for VPD was comparable to that reordered on different grapevine cultivars (1.5 kPa)¹⁹. The values of g_{smax} and C_{imax} are almost equal (Table 1). The g_{smax} and C_{imax} that recorded in cultivated *S. persica* were about 60 μmol H₂O m⁻² sec⁻¹ and 200 μmol H₂O m⁻² sec⁻¹ by Rangani *et al.*²⁰.

The PAR was the most important ecological factor that affecting photosynthesis rate, followed by air CO₂ concentration²¹. The net photosynthesis rate values for drought treatments in the morning, when the C_i levels was 350 μmol mol⁻¹ as reported by Xiao *et al.*²². This result supports our assumption that *S. persica* grows well at the study area in Rabigh; as our data revealed that C_i was 303.00 μmol mol⁻¹. This means that A went at maximum rate. The data in Table 1 shows that A and E values were 53.96 μmol m⁻² sec⁻¹ and 6.44 mmol m⁻² sec⁻¹, respectively. Both values are much higher than that for the cultivated *S. persica*²⁰.

The WUE represents the ratio of biomass produced to water used. It is thought to be a relevant parameter in determining crop productivity, when water is limiting²³. The WUE is correlated with the canopy leaf structure



Fig. 2(a-f): Photos for green bushes of *Salvadora persica* grown at Rabigh Province, Saudi Arabia during March-April 2016 and 2017

Table 1: Parameters indicate the physiological status of *Salvadora persica* plants grown at Rabigh Province, Saudi Arabia during March-April, 2016 and 2017

Parameters	Values
Leaf area (cm ²)	4.71 ± 2.05
Leaf temperature (°C)	33.28 ± 3.0
VPD _{minimum}	2.74 ± 0.2
VPD _{maximum}	3.10 ± 0.05
g _{s minimum}	157.66 ± 15.17
g _{s maximum}	336.60 ± 74.39
C _{i minimum}	64.00 ± 1.630
C _{i maximum}	303.00 ± 53.24
A _{minimum}	16.13 ± 6.360
A _{maximum}	53.96 ± 10.17
E _{minimum}	3.77 ± 0.75
E _{maximum}	6.44 ± 1.77
WUE _{minimum}	3.33 ± 1.61
WUE _{maximum}	7.89 ± 0.67
PAR _{minimum}	155.00 ± 21.65
PAR _{maximum}	219.33 ± 17.82
CAT	3.58 ± 0.785
Trehalose content (µg g ⁻¹ dry weight)	4.33 ± 2.78

Values in the table are means of 5 plants during the 2 seasons, ±: Standard deviation, minimum value was recorded on old foliage leaf, while the maximum value was recorded on new foliage leaf of *S. persica*, VPD: Vapor pressure deficit (kPa), g_s: Stomatal conductance to water vapor (µmol H₂O m⁻² sec⁻¹), C_i: Substomatal CO₂ (µmol mol⁻¹), A: Net photosynthesis rate (µmol m⁻² sec⁻¹), E: Net transpiration rate (µmol m⁻² sec⁻¹), WUE: Water use efficiency (mmol CO₂ mol⁻¹ H₂O), PAR: Photosynthetic active radiance (µmol m⁻² sec⁻¹), CAT: Catalase activity (mM H₂O₂ g⁻¹ fresh weight min⁻¹)

and works as a consequence of both changing environmental conditions and the physiological changes expected with leaf aging. It modifies leaf photosynthesis and transpiration. From literatures, the measured WUE in field-grown Grenache and Tempranillo plants, changed from 40-80 µmol CO₂ mol⁻¹ H₂O²⁴. These values are much less that we recorded in our study area for *S. persica* (Table 1).

Organic osmolytes are small solutes used by cells of numerous water-stressed organisms and tissues to maintain cell volume. Trehalose, is one of these osmolytes. Trehalose has been detected in a wide range of organisms and possesses many biological functions ranging from serving as an energy source to acting as a protective and/or signal sugar against abiotic stress. Its synthesis in plants is catalyzed by trehalose-6-phosphate synthase (TPS) and trehalose-6-phosphate phosphatase (TPP)²⁵. The biosynthesis pathway in higher plant had been well established but, due to the rapid degradation of trehalose by trehalase, trehalose does not accumulate in most plant species²⁶:

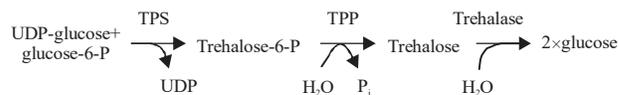


Table 2: Analysis of total nitrogen and some chemical ions in *Salvadora persica* plant shoot grown at Rabigh Province, Saudi Arabia during March-April, 2016 and 2017

Ions	mg g ⁻¹ Dry weight
Total N	165.000±35
PO ₄ ³⁻	0.600±0.15
K ⁺	6.550±1.08
Na ⁺	4.940±2.61
Ca ²⁺	31.250±1.5
Mg ²⁺	3.380±1.07
Zn ²⁺	0.005±0.001
Fe ²⁺	0.145±0.02
Si ²⁺	0.055±0.03
MoO ₄ ²⁻	>0.005

Values in the table are means of 5 plants during the 2 seasons, ±: Standard deviation

Table 3: Analysis of soil rhizosphere around *Salvadora persica* grown at Rabigh Province, Saudi Arabia in March-April during 2016 and 2017

Parameters	Values
Relative humidity (%)	33.00
CO ₃ ²⁻ (mg g ⁻¹ dry wt)	5.75±2.25
TDS (mg g ⁻¹ dry wt)	26.50±0.5
Ec (mS cm ⁻¹)	48.00±5.0
pH	7.80
Ions	mg g ⁻¹ Dry weight
PO ₄ ³⁻	0.77±0.14
K ⁺	0.63±0.001
Na ⁺	0.860±0.42
Ca ²⁺	8.830±3.9
Mg ²⁺	5.500±0.4
Zn ²⁺	0.030±0.01
Fe ²⁺	22.08±7.92
Si ²⁺	0.200±0.01
MoO ₄ ²⁻	<0.0005
Cl ⁻	3.500±0.13

Values in the table are means of 5 plants during the 2 seasons, ±: Standard deviation

In this study, the trehalose content in the plant shoot was 4.33 µg g⁻¹ dry weight. No available review has been reported on trehalose content in *S. persica* shoot or other desert plants. Few reports were demonstrated on cultivated tobacco plant and castor bean leaves with 1.14 and 0.96 mg g⁻¹ fresh weight trehalose, respectively. Trace amount of trehalose (0.02-0.09 mg g⁻¹ fresh weight) was detected in banana and sweet potato leaves²⁶. Trehalose can protect structural and functional proteins from denaturation by high temperatures. This proves that its presence helps the plants to survive and grow well under a biotic stressed conditions. This proven is also convenient and supported with the presence of catalase activity which is an antioxidant enzymes, usually elevate in the cell to help plant in facing the stress conditions. The antioxidants included enzymatic antioxidants (e.g., superoxide dismutase, peroxidase and catalase) and non-enzymatic antioxidants (e.g., ascorbic acid, α-tocopherol, glutathione, carotenoids and flavonoids²⁷. Antioxidants may

help the plants to protect it against various types of oxidative damage caused environmental stresses. So, the activity of antioxidant enzymes positively coordinates with the surrounding stress conditions. As they keeps the membrane and machinery compounds in the living cell. Rangani *et al.*²⁰ reported on the presence of CAT activity in *S. persica* under salinity stress conditions. In this study, catalase activity in *S. persica* shoots (Table 1) is much less than that recorded by Mohamed and Khan²⁸ (2.5 unit g⁻¹ tissue). The data revealed that the *S. persica* plants in Rabigh area is growing and developing well, as the activity of CAT is not jump over the average level of that enzyme in plant cells.

Salt accumulation in the root zone has great agricultural impact that can affect the plant growth, yields and tolerance to stresses. As well as the composition of cations on the exchange complex of soil particles, this subsequently influences soil permeability.

Table 2, contains the values of chemical ions analyzed in the *S. persica* shoots. The nitrogen content was 165 mg g⁻¹ dry weight. Nitrogen is a key factor which affecting the leaf area, photosynthesis rate, growth and development of the plant. Theoretically, the substantial amount of N requires for plant growth is 1000 µg kg⁻¹ dry weight²⁹.

The Ca²⁺ represent the highest divalent compounds in *S. persica* shoots. However, its presence in the soil is not so high as in the plant shoot (Table 2, 3). So its accumulation in plants supports the cell wall coherence and helps in keeping the plant grow in this area. There is a balance between the amount of K⁺ (6.55 mg g⁻¹ dry weight) and Na⁺ (4.94 mg g⁻¹ dry weight) in the plant shoot (Table 1). The K⁺ is a major plant macronutrient that plays important roles related to stomatal behavior, osmoregulation, enzyme activity, cell expansion, neutralization of non diffusible negatively charged ions and membrane polarization. The K⁺ concentrations are also closely related to drought resistance³⁰.

The Na⁺ maintains the osmotic potential in the cells³¹. Insufficient Na⁺ disturb intracellular ion homeostasis which leads to membrane dysfunction, attenuation of metabolic activity and secondary effects that cause growth inhibition³².

Regard the soil analysis, there are a variety of techniques to measure soil salinity including, the measurement of the mass of total dissolved solids (TDS, mg L⁻¹) or the electrical conductivity (EC, dS m⁻¹) of soil water extracts, or the proportion and composition of salt species using spectrophotometry³³. Soil EC has become one of the most reliable and frequently used measurements, particularly to characterize field variability. Table 3 summarized the results of carbohydrate content, total dissolved soluble (TDS), EC and pH. Soil contains large amounts of carbonates that reflect its

alkaline pH (7.8). The coastal soil has a high EC (85 mS cm⁻¹), however, the mangrove soil has an EC (50.7 mS cm⁻¹) or desert soil has a very low EC (2.0 mS cm⁻¹)³⁴. Abd El-Salam and Elhakem³⁴ reported that Coastal soil has a high concentration of TDS (550 mg L⁻¹), however mangrove soil has soluble salts concentration (125 mg L⁻¹) or desert soil has a very low TDS amounted as 83.4 mg L⁻¹. Although our data for TDS was lower than that in the literature, but there is no remarkable effect of it on the growth of *S. persica* in the studied area.

Four major exchangeable cations (i.e., Ca²⁺, Mg²⁺, K⁺ and Na⁺) and major anions (i.e., Cl⁻ and CO₃²⁻) in the soil solution and the precipitated salts CaCO₃ (lime) usually determine the soil fertility. The data in Table 3 revealed that, the highest ions content was recorded for Fe²⁺ (22.08 mg g⁻¹ dry weight). The other elements, Mg²⁺, Zn²⁺, Si²⁺ and MoO₄²⁻ presents in very trace amounts. Iron is the fourth most abundant element on earth and soil typically contains 1-5% total iron³⁵. Most iron in the soil is found in silicate minerals or iron oxides and hydroxides, forms that are not readily available for plant use³⁶. Lack of Fe reduced the formation of thylakoid membranes in chloroplast³⁷. Physiological parameters, such as chlorophyll and CO₂ gas exchange measurements, can substantiate the tolerance of plants to Fe deficiency or high pH conditions³⁸. In this study, the level of Fe²⁺ in the soil indicates that the plants did not suffer from iron deficiency and the rate of photosynthesis is high in comparing with that reported by Incesu *et al.*³⁵.

These finding will persuade and encourage the cultivation of *S. persica* plants in Rabigh. This will output an economic, social and environmental impacts, especially sand occupy more than a quarter of Saudi Arabia area.

CONCLUSION

Investigations put a spot on the ability of cultivation of *S. persica* in Rabigh Province to grow and develop in these areas which help in fixing sands. Fixation of sand represents one of the glory goals for Saudi Arabia as it plays a real role in economy and environment development. The physiological state of the plant in this study area has been studied by measuring leaf area, VPD, C_i, g_s, photosynthesis, transpiration rates, WUE and PAR, as well as the trehalose level and CAT activity. The macroelements and microelements contents were in sufficient level in plant and soil. All of these results persuade that *S. persica* plant grows well in this area, hence we can encourage its planting in this area in order to fix the sand in Rabigh Province, Saudi Arabia.

ACKNOWLEDGMENT

The deep grateful is sending to Professor Hanan Ibrahim and Professor Enas Abutaleb, Department of Water Pollution Research, National Research Center, Egypt for using instruments required for plant and soil chemical analyses. Sincere thanking is for Mr. Mubark Aljeddani and Mr. Basam Elsharmarany for helping in plant and soil collections.

SIGNIFICANCE STATEMENT

This study discovered the ability of *S. persica* to grow will under dry condition that can be beneficial for sand fixation, specially Rabigh area is unused and wide dry sand area. These findings will open the door for attempts to planting this area, may be with another edible or more economic plants.

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