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SOME LEAF CHARACTRISTICS OF *SALVADORA PERSICA* L. (FAMILY SALVADORACEAE) FROM FRINGES OF SUPRA-LITTORAL ZONE OF SANDS PIT (HAWKES BAY), KARACHI, PAKISTAN

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ABSTRACT

Some morphometric and micromorphological characteristics of *Salvadora persica* L. leaves are described from a plant growing at the fringes (often inundated with Seawater during high tides) of supra-littoral salt marsh of Sands pit, Karachi. Leaf length of *S. persica* averaged to 5.23 ± 0.1045cm (2.4 to 7.8 cm, CV: 20.06%) and breadth to 2.54 ± 0.051cm (0.9 to 3.70, CV: 19.98%). Leaf area measured (LAMG) averaged to 9.5150 ± 0.3150 (CV: 33.24%). *S. persica* showed simple dicotyledonous anatomy with palisade tissue on the both surfaces. Leaf area was best estimated arithmetically by employing multiplying factor (K = 0.7015) in equation LAK = (LL x LB) x K. The lamina weight per leaf averaged to 0.1749 ± 0.0066g and (174.90 ± 0.660mg) varying around 37.9% and tended to follow normal distribution. Lamina weight tended to be positively correlated with LAMG as given below –

Ln Lamina weight (mg) = 18.517 + 0.994 (Ln LAMG, cm^2) ± 0.217; r = 0.887, F = 366.3 ($p < 0.0001$)

SLA of *S. persica*, in a sample of 101 differentially aged leaves averaged to 56.69 ± 1.167cm^2/g corresponding to 5.67 ± 0.1167$m^2.kg^{-1}$. It varied from 37.60 to 97.27 cm^2/g (CV: 20.74%) that is 3.76 to 9.73 $m^2.kg^{-1}$.

Leaves were amphistomtatous. Stomata were oval in shape and ledges arched. Stomatal types included paracytic, tetracytic, staurocytic, anisocytic and anomocytic stomata. Stomatal density /mm^2 averaged 218.14 ± 3.64 on dorsal surface and 241.99 ± 3.057 on ventral side. Stomatal size averaged to 16.32 ± 0.508 x 10.71 ± 0.292 µm on dorsal side and 17.01 ± 0.472 x 11.91 ± 0.284 µm on ventral side.

Key Words: *Salvadora persica* L., Leaf characteristics, Leaf area estimation and Stomatal types.

INTRODUCTION

Salvadora persica L. is commonly known as Meswak, Peelu, or Mustard tree, etc. In the premises of WWF Wetland Centre (Pakistan) Karachi, *S. persica* was observed growing in remarkably close neighborhood of *Avicennia marina* and *Arthrocnemum indicum* in the fringes of supra-littoral zone of Sands pit (often inundated with Seawater during high tide), Hawkes Bay, Karachi (Fig. 1). It is a sodiophillic (Hadi *et al.*, 2018) micro-phanerophytic plant of dense canopy, producing bunches of flowers and red small fruits. Large communities dominated by *S. persica* may be found in water-logged halocatena of River Indus flooded areas of Hyderabad district, often forming large phytogenic mounds (Khan *et al.*, 2003). It can withstand salinity of 50 dSm^{-1} (Qadir *et al.*, 2008) and establishes with profuse branches in black soil with salinity > 60 $dS.m^{-1}$ (Rao *et al.*, 1999). Its anti-oxidative enzymes protect it against salinity (Rangini *et al.*, 2016). Mepham and Mepham (1985) have reported this species from East African region, shore of Red Sea or islands of the Indian Ocean and regarded it as a potential mangrove species. *S. persica* contains abrasives, antiseptics, astringent, detergents, enzyme inhibitors and fluoride. It is favourite fodder for camels. It has anti-urolithiatic properties (Geetha *et al*., 2010). Chemical composition of *S. persica* is given by Kamil *et al.*, 1999 a, b, c; 2000). Root and shoot sticks are used as oral hygiene brushes in many parts of the world (Evenari and Gutterman, 1973). *S. persica* is anti-microbial and anti-cariogenic (Halawany, 2012) and antidiabetic (Yadav and Saini, 2009). Its bark and fruits contain saponins and fish poison (Gurudeeban *et al*., 2012). Its volatile oils show activity against *Pseudomonas aeruginosa* and *Staphylococcus aureus (*Alali *et al*., 2009*).* This paper describes some characteristics of leaves of this plant growing in wet fringe area of supra-littoral zone of Hawkes Bay, Karachi, Pakistan.

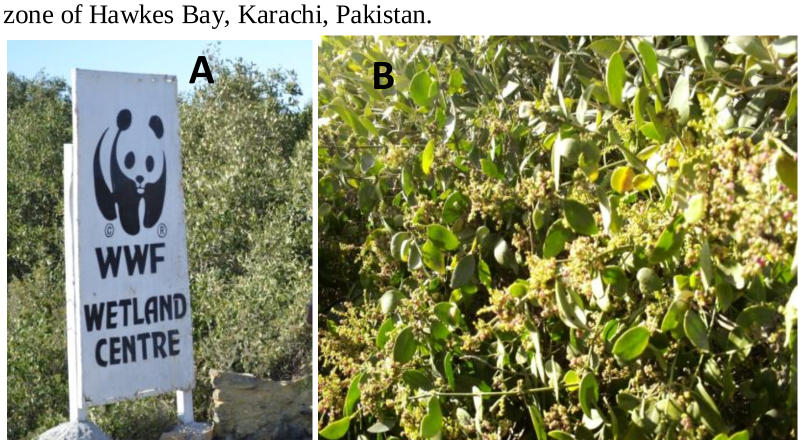


Fig. 1. A sward of *Salvadora persica* and *Avicennia marina* in the coastal wetland of Sands pit (A) and a close up view of *S. persica* (B). The area is inundated with Seawater during high tide.

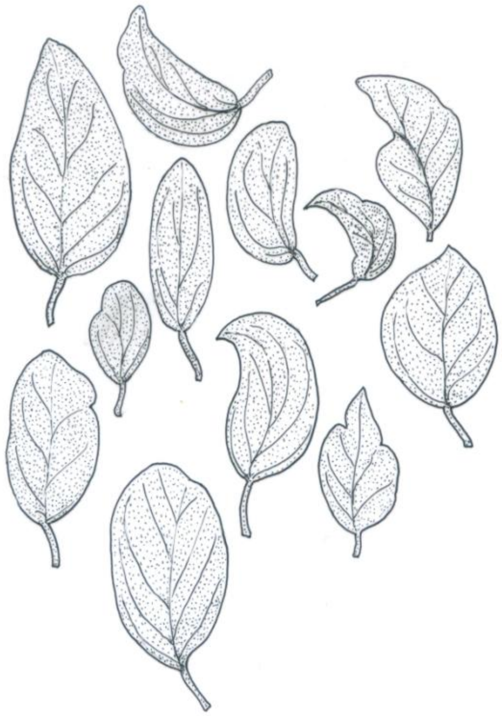


Fig. 2. Variously shaped leaves of *S. persica* collected from a single tree growing in the wetland in Sands pit in the premises of WWF, Pakistan. The plant was inundated with Seawater during high tide. Leaves drawn not to the scale.

Physico-chemical properties of Seawater

The physico-chemical properties of Seawater of Sindh coast are given in Table 1. Seawater is basic in reaction pH: up to 8.5 but much variable (6.4 to 9.6) in Bakran area probably due to industrial discharge. Mildly basic in Karachi Harbour estuarine ecosystem (7.3-8.1). Temperature is low in winter and high in summer (24.8 -30.0 ^oC). Salinity is high. It varies around 40 $dS.m^{-1}$ but has also been recorded as high as 51.4-55.3 $dS.m^{-1}$ in Korangi creek. TDS is low in winter months and high in June.

MATERIALS AND METHODS

The plant material for this study was collected from the wetland of the Sands pit, Hawkes Bay, Karachi. This area is often inundated with Seawater during high tides. One hundred and one leaves were randomly collected from a twig of this plant and they were recorded for their length of petiole (PL), lamina length lying between the point of midrib insertion and the apex of the leaf (LL) and lamina breadth (LB) at the broadest points. Hickey (1979) and LWG (1999) were followed for description of leaf.

Table. 1. Physico-chemical properties of Seawater along Sindh Coast.

Place	pH	Temp (^{o}C)	EC ($dS.m^{-1}$)	PPT	TDS / TSS
			Salinity		
Karachi Harbour! (Estuarine Ecosystem)	7.3-8.1	19.8-21.05	-	31.6-36.0	Total suspended solids (TSS: 122-166 mg. L^{-1}
Korangi creek ***	7.3-7.9	Low (17.5 – 20 ^{o}C) in Dec. to Feb. and relatively higher (24.8 -30 ^{o}C) in summer (Apr- Oct.) **	51.4-55.3	28* 39-41***	Ambrah Creek – TDS low in Oct. – Feb. and high in June **
Khudi creek	-		-	30 *	
Bakran ****	6.4 - 9.6		-		
Keti Bunder	-		-	41*	
Shah Bunder	-		-	39-41*	
Clifton ***	8.0 - 8.2		50.9-55.6	37-41	
Manora ***	8.2 - 8.5		43.7-50.8	28-36	
Indus delta***	7.3 - 8.3		46.6-56.6	32.45	

!, Ali *et al.* (2012), *, IUCN – Pakistan; **, Mahar and Awan (2012) – Low TDS' 10,000 – 40,000 mg / L and high TDS, reaching up to 90,000 mg /L. The dissolved O_2 as per this data relates inversely with TDS (r = - 0.617). ***, Qureshi *et al* (2000); ****, Siddiqui and Qasim (1990).

To determine the leaf area, the leaf outline was carefully drawn on graph paper and area (LAMG) determined with all possible precision and accuracy. The multiplication factor (K) was calculated by employing the formula, K = Area measured / (LL x LB). Employing average value of the multiplication factor K, leaf areas were also calculated as Leaf Area computed (LAK) = K (length x breadth) for comparison with the observed areas (LAMG) of the leaves. Bivariate power relationship of leaf area with measured linear dimensions of the leaf were computed and expressed as LAPOW. In addition to it, leaf area (LMULTI) was computed with the help of the regression coefficients determined by employing multiple regression method fitting in the allometric model, $Y = a + b_1LL + b_2 LB \pm SE$.

For dry weight determination, leaves were kept continuously in oven at 70 ^oC for two days and then weighed. Specific Leaf Area (SLA) was expressed as the ratio of one-sided leaf area (Westoby *et. al.*, 2000) to dry leaf mass. Specific leaf mass (SLM) was equal to SLA^{-1}.

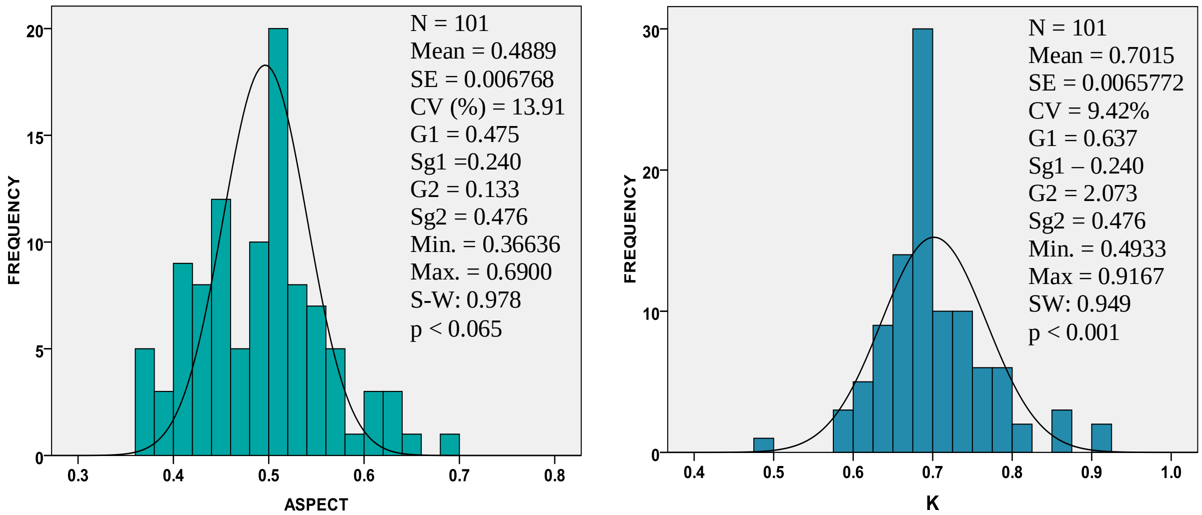


Fig. 3. Frequency distribution of aspect ratio (LB / LL) and coefficient K for 101 leaves of *S. persica*.

Table 2. Morphometric and architectural parameters of *S. persica* leaves collected from a naturally growing plant from wetland of Sands pit, Hawkes Bay, Karachi (N = 101 except petiole and leaf weight with N = 85).

Parameters	N	Mean	SE	CV%	g1	g2	Min	Max	S-W	p	Curve
LL (cm)	101	5.2337	0.10445	20.06	-0.391	0.206	2.40	7.80	0.985	0.292	S
LB (cm)	101	2.5371	0.05045	19.98	-0.630	0.524	0.90	3.70	0.969	0.016	AS
LAMG (cm^2)	101	9.515	0.31497	33.24	-0.168	0.105	1.48	18.75	0.985	0.320	S
AA (^{o})	101	67.28	0.970	14.49	0.918	1.845	48	104	0.948	0.001	AS
BA (^{o})	101	82.88	0.879	10.65	0.235	1.501	55	115	0.977	0.070	S
PL (cm)	101	1.1119	0.0280	25.32	0.167	-0.240	0.50	1.80	0.977	0.079	S
Petiole wt (g)!	85	0.0053	0.00022	38.64	0.238	- 0.172	0.0011	0.1050	0.984	0.376	S
Lamina wt. (g)	101	0.1749	0.0066	37.92	0.112	-0.080	0.0158	0.3463	0.994	0.937	S
Leaf weight (g)	85	0.1804	0.007311	37.36	0.072	-0.076	0.0169	0.3474	0.995	0.981	S
Aspect ratio*	101	0.48895	0.00676	13.91	0.475	0.133	0.3636	0.6900	0.978	0.065	S
K	101	0.70152	0.00657	9.42	0.637	2.073	0.4933	0.9167	0.949	0.001	AS
LAK (cm^2)	101	9.5149	0.31496	33.27	-0.168	0.105	1.48	18.75	0.985	0.320	S
LAPOW (cm^2)	101	9.4863	0.30680	32.50	-0.333	0.351	1.654	16.272	0.980	0.129	S
LMULTI (cm^2)	101	9.4297	0.29539	31.43	-0.663	0.041	1.1055	15.089	0.957	0.002	AS
SLA (cm^2/g)	101	56.694	1.16976	20.74	1.093	1.410	37.598	97.271	0.930	0.000	AS
SLM (g/cm^2)	101	0.01832	0.00035	18.76	0.008	-0.175	0.0103	0.0266	0.993	0.903	S

LL, Lamina length (cm); LB, Lamina breadth (cm); LAMG, Lamina area measured graphically; AA, Apex angle; BA, Base; angle; PL, petiole length; Aspect ratio, LB / LL; LAK, Lamina area measured through K; LAPOW, Lamina area estimated through power model equation; Lmulti, Lamina area estimated through multiple linear equation, SLA, Specific lamina area; SLM, Specific lamina mass. For N = 101, SE of skewness (Sg1) = 0.240 and SE of kurtosis (Sg2) = 0.476**,** N = 85 (petiole weight), Sg1 = 0.261; Sg2 = 0.517. S-W, Shapiro-Wilk (1965) test of normality. S, Symmetrical; AS, Asymmetrical.

To study mature stomatal types, leaf epidermal impressions were made with clear nail polish (Wang *et al.*, 2006) and studied under compound optical microscope. For scanning electron microscopy (SEM), air-dried leaf was mounted on brass stubs and coated with a 250 ^{o}A gold layer with JFC-1500 gold coater. Scanning Electron Micrographs (SEMs) were obtained at 15kV with JEOL JSM-6380A electron microscope at various magnifications. The images were saved digitally on computer. Stomatal nomenclature suggested by Prabhakar (2004) being simple and based upon structure of stomata and not their ontogeny pathways was adopted to ascertain types of the mature stomata. This classification does not differentiate between distinct and indistinct neighbouring cells abutting stomata. Indistinct neighbouring cells are considered as important as distinct cells owing to their abutting position. Stomatal density was determined microscopically at 45 x 10 X magnification. Measurement of stomatal size was made through calibrated ocular scale on the basis of nail polish imprints of the leaf surfaces. The data was analyzed statistically (Zar, 2010). Normality of distribution was tested using Shapiro-Wilk's (1965) test.

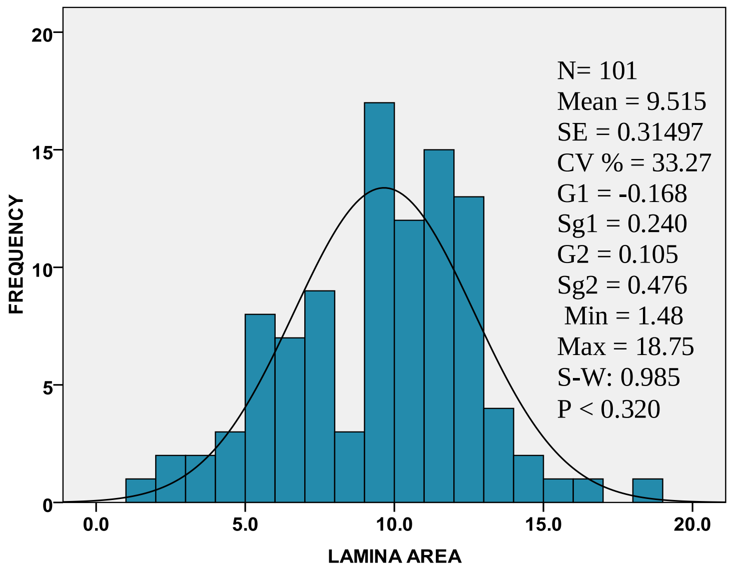


Fig.4. Frequency distribution of lamina area (LAMG, cm^2) of S. *persica* leaves.

RESULTS AND DISCUSSION

Leaf morphometric and architecture

The morphometric and architectural parameters of *S. persica* leaf are presented in Table 1. Leaves are thick succulent ovate-lanceolate, dorsiventral, and opposite-decussate. They are green, relatively thick, brittle and occasionally varying in shape (Fig. 2). The leaves are shortly petiolate, exstipulate, entire-margined and pinnately veined. Midrib is prominently present on ventral surface with a hump near umbo. Trichomes are rare on the veins only.

Petiole: Petiole is small, cylindrical, and green. The petiolar length averaged to 1.11 ± 0.028 cm, 0.5-1.8 cm) (Table 1). In 93.1% of the leaves petiole varied from 0.5 to 1.5 cm in length. Petiole weight averaged to 5.32 ± 0.028mg varying from 1.1 to 10.5mg; CV: 38.64%). In around 79 % of the leaves, petiole weight varied between 2.6 to 7.5mg.

Petiolar insertion with lamina is interesting. Petiole is generally inserted at 180^o with the lamina but at times obliquely (at various angles) in the umbo and even occasionally putting lamina at almost right angle to the petiole. This strategy appears to facilitate leaf adjust to sun in the dense canopy of the plant. The leaf orientation to sun should obviously be a crucial adaptation with dense bushy habit.

Leaf Length, breadth and aspect ratio: Leaf length of *S. persica* averaged to 5.23 ± 0.1045cm (2.4 to 7.8 cm, CV: 20.06%) and breadth to 2.54 ± 0.051cm (0.9 to 3.70, CV: 19.98%). Around 38.6% of the leaves had length 2.6 to 5.0cm. Some 56.4% of leaves were 5.1 to 7.0cm in length. Most of the leaves (72.3%) were 2.1 to 3.0cm in breadth and only 10.9% were larger (3.1 – 4.0 cm) in breadth.

In present investigation, the leaf shape consistency was determined as Aspect ratio = Breadth / Length of leaves (Fig. 3). This parameter may give some indication about consistency of leaf shape with size (Verwijst and Wen, 1996). Aspect ratio (LB / LL) averaged to 0.489 ± 0.0068 (CV = 13.9%). Aspect ratio in 84.2 % leaves ranged between 0.41 to 0.6. It exhibited significant correlation with leaf length ($r = 0.309$, F: 10.467, $p < 0.002$) and leaf breadth ($r = 0.364$, F: 15.456, $p < 0.0001$) i.e. Leaf Length had the explanatory power of aspect ratio variation little lower (9.55%) than the leaf breadth (13.25%). Aspect ratio, however, exhibited no correlation ($r = 0.005$, F: 0.003, $p < 0.958$) with measured leaf area (LAMG). Leaves, therefore, appeared to exhibit considerable consistency of shape with age. The form of few leaves was somehow deformed due to some unknown reason (s).

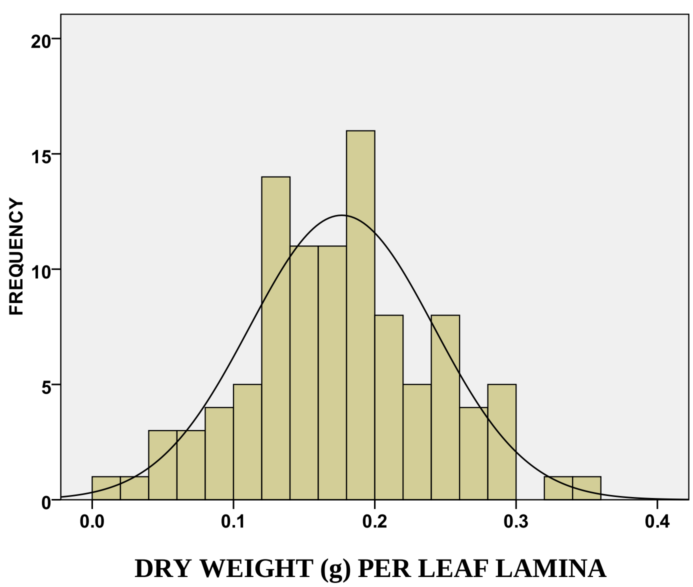
The multiplicative factor K: The values of multiplicative factor (K) of the equation K = Area / (LL x LB), tended to be leptokurtic in distribution (Fig. 3) and averaged to 0.7015 ± 0.0066 varying only 9.42% only. Distribution of K tended to be somewhat positively skewed and around 89% of the K values were found to be between 0.6 and 0.8.

Apex and Base angles: Both apex and base angles averaged to be lower than 90^o. Mean base angle was, however, larger (82.9^o, 55 – 105^o) than the apex angle (67.3^o, 48 -104^o). Apex angle was lesser than right angle in 98% of the leaves; only 2% of the leaves had apex angle greater than the right angle. Apex angle was thus predominantly acute. The base angle was lesser than right angle in 83.2% leaves and higher than the right angle in 16.8%. That is proportionately larger number of leaves had obtuse base but acuteness of base was still apparent in majority of leaves. It followed that leaves were ovate to elliptical and fairly consistent in form.

Table 3. Correlation and regression analyses for lamina area estimation in *S. persica*.

Simple Linear Correlation and Regression
LAMG (cm^2) = 0.518 + 0.658 (LL x LB, cm) ± 0.828, R = 0.966; R^2 = 0.932; Adj. R^2 = 0.953; F = 1363.2 t = 2.015 t = 36.93 p < 0.047 p < 0.0001--Eq. # 1,
Multiple Correlation & Regression
LAMG (cm^2) = - 6.638 + 1.706 LL + 2.807 LB ± 0.899, R 0.960; R^2 = 0.921; Adj. R^2 = 0.919, F = 570.77 t = -13.64 t = 12.92 t = 10.41 p < 0.0001 p < 0.0001 p < 0.0001 ---Eq.# 2. Zero Order r Partial r LL 0.913 0.794 LB 0.887 0.725
Power model
LAMG (cm2) = 0.797. $(LL \times LB)^{0.948}$ ± 0.091, R = 0.976; R^2 = 0.953; Adj.R^2 = 0.953; F = 2014.77 t = 18.38 t = 44.89 p < 0.0001 p .0001 --Eq.# 3 (Best fit).

LAMG, Lamina area measured graphically (cm^2) for a leaf; LL, Lamina length (cm); LB, Lamina breadth (cm); LL x LB, Multiplicative parameter of length and breadth.



N = 101
Mean = 0.1749
SE = 0.006599
CV % = 37.92
G1 = 0.112
Sg1 = 0.240
G2 = 0.0800
Sg2 = 0.476
Min. = 0.0158
Max = 0.3463
S-W: 0.994
P < 0.937

Fig. 5. Frequency distribution of lamina dry weight (g) of *S. persica* leaves. *S. persica* is a true halophytic plant with succulent leaves which become gradually thicker with age as they are employed as the salt sink by the plant. On maturity they turn pale and shaded from the plant. S-W: Shapiro-Wilk test.

Lamina area: Lamina area was determined graphically and referred to as LAMG, presented in Fig. 4. The location and dispersion parameters of lamina areas determined through various methods are presented in Table 2. LAMG, LAK, LAPOW and LMULTI averaged to 9.5150 ± 0.3150 (CV: 33.24%), 9.5149 ± 0.31496 (CV: 33.27%), 9.4863 ± 0.3068 (CV: 32.50%) and 9.4290 ±0.2954 (CV: 31.435) cm^2, respectively. On the basis of Shapiro-Wilk test all leaf area parameters except LMULTI tended to follow normal distribution.

Table 3 presents the results of correlation and regression analysis for lamina areas estimation. All the three equations based on 1) simple correlation and regression of LAMG (Y) with a multiplicative parameter LL x LB as X, 2) Multiple correlation and regression analysis of LAMG (Y) with LL and LB as independent X variables and 3) Power model regression of LAMG (Y) with LL x LB as X, were statistically significant in terms of r, R, F, and t-values. Amongst these three equations, power model equation was the best fit equation for the estimation of leaf area statistically. Many workers have undertaken leaf area estimation allometrically as well as mathematically and have arrived at significant results with many species (see Khan, 2008; 2009 for details). The fitness of power model to estimate leaf blade area has been reported in several species e.g., in *Coffea arabica* and *C. canephora* with high precision (R^2 = 0.998) and accuracy irrespective of cultivar and leaf size and shape (Atunes *et al.*, 2008), in 'Niagara' (R^2 = 0.992) and 'DeChunac' (R^2 = 0.963) grapevines (Williams III and Martinson, 2003); groundnut (Kathirvelan and Kalaiselvan, 2007) and *Nicotiana plumbaginifolia* (Khan, 2008).

Relationships amongst measured and estimated lamina areas: The average values of various leaf area parameters although appeared to be quite comparable to each other, the superiority of estimation methods needed to be tested. When tested through correlation analysis, the estimated leaf areas (LAK, LAPOW and LMULTI) were highly correlated amongst themselves and with LAMG (Table 4) but LAMG AND LAK were most closely related (r = 0.999) which implied that estimation of leaf area on the basis of LL and LB using K as multiplicative factor (0.7015) is the most suitable in *S. persica*. Ahmed and Khan (2011) have also recommended the arithmetic method of using K as multiplication factor to be most accurate in *Jatropha curcas* (with a value of K = 0.858758). Besides being accurate it is simple and convenient also. This contention was further substantiated on the basis of composition similarity determined via Czekanowski's (1913) index of similarity on the basis of % frequency of occurrence of leaf area values in 20 classes of equal class size interlude. Composition similarity was 90% between LAMG and LAPOW, 92.8% between LAMG and LMULTI and maximum (97.6%) between LAMG and LAK. The estimation in leaf area on the basis of multiplicative factor K (0.7015) may, therefore, be recommended owing to its precision, simplicity and convenience.

Table 4. Values of linear correlation coefficients among variously determined leaf areas.

LAMG	LAMG			
LAK	0.999	LAK		
LAPOW	0.966	0.966	LAPOW	
LMULTI	0.961	0.961	0.993	LMULTI

Lamina dry weight: The lamina weight per leaf averaged to 0.1749 ±0.0066g and (174.90 ± 0.660mg) varied around 37.9% and tended to follow normal distribution (Shapiro-Wilk test: 0.985, p < 0.320; Table 2, Fig. 5). Lamina weight of 72.2% of the leaves ranged between 0.11 to 0.25g. Lamina weight tended to be positively correlated with LAMG as given below:

$$\text{Log}_e \text{ Lamina weight (mg)} = 18.517 + 0.994 (\text{Log}_e \text{ LAMG, cm}^2) \pm 0.217$$

t = 8.668 t = 19.139

P < 0.0001 p < 0.0001;

r = 0.887, r^2 = 0.878, F= 366.3 (p < 0.0001)

Petiole / lamina weight ratio (PLWR): PLWR ratio was determined as ratio of Petiole weight to Lamina weight expressed in percent. It averaged to 3.5005 ± 0.2117 varying from 0.32 to 10.10%, CV: 55.75%). The parameter tended to not distribute normally (Shapiro-Wilk test: 0.915, p < 0.0001).

This parameter was below 2% in 17.6 % of the sample leaves. It ranged from 2.1 to 4.0 in 53% of the leaves. Some 15.3 % of the leaves had PLWR between 4.1 to 6.0%. PLWR ratio was ≥8 in 14.1 of the leaves. This variation may obviously be attributed to the leaf size variation and the physiological state of the leaves. In present studies, PLWR was significantly negatively linearly correlated with LAMG. That is larger the leaf, smaller is petiole weight to lamina weight ratio. Variation in lamina weight accounted for variation in PLWR around 43.4%.

$$\text{PLWR} = 7.189 - 0.389 \text{ LAMG} \pm 1.477$$

t = 14.83, t = -7.973

p < 0.0001 p < 0.0001

r = 0.659, r^2 = 0.434, F: 63.56 (p < 0.0001)

Leaf weight: The leaf dry weight (sum of lamina and petiole weights) averaged to 180.43 ± 7.31 mg (0.1804 ± 0.0073g) varying from 169.0 to 347.4 mg (CV: 37.35%) (Table 2). Leaves with ≤ 125.1 mg were 16.5%, those ranging between 125.1-250.0mg in weight were 67.0% and heavier leaves (250.1 to 325.0 mg were 14.1%. Very heavier leaves were only 2.4% of the total leaves. The leaf weight tended to follow normal distribution (Shapiro-Wilk test: 0.995, p < 0.981).

Internal structure of petiole and leaf: TS of petiole (Fig. 6 and 7) was circular in cross section outline. It is bordered with single layer of epidermis covered with thick cuticle. Epidermis is stomatiferous. Epidermal cells are small, squarish-rectangular. Cortex is compact multi-layered (c. 12 cell layers) with small intercellular spaces. The cortical cells are thin-walled, parenchymatous, round to irregular in shape. The size of cortical cells increases approaching pericycle. Pericycle is sclernchymatous and discontinuous. Endodermis single layered. Vascular bundle (VB) is round in the centre of the petiole with thicker rim of xylem. Phloem is external to xylem but intraxyllery phloem patches are present distributed randomly in the xylem tissue. The lumen of vessels is generally round. Fig. 8 presents the TS of leaf from midrib region including some part of lamina wings as well. Leaf presents the structure of common dicotyledon plant- a non-C4 type anatomy. Mesophyll contains crystals druses. Lamina margins entire. The epidermis is covered with thick cuticle. Hypodermis is present on dorsal surface. Palisade tissue is present on both surfaces, adaxial and abaxial. Palisade tissue is continuous in the midrib region dorsally but discontinuous in ventral midrib region. Pericycle is variably multilayered and discontinuous. There occurs large vascular bundle in the midrib and several irregularly distributed small VBs in lamina wings due to reticulate venation of leaf. Cortical cells are parenchymatous similar to those of the petiole. The corner of the lamina wings is characterized with multilayered hypodermis (Fig. 9A). Stomata are present on both leaf surfaces. Foliar sclereids are present. The clustered crystals may be seen in some cells of cortex.

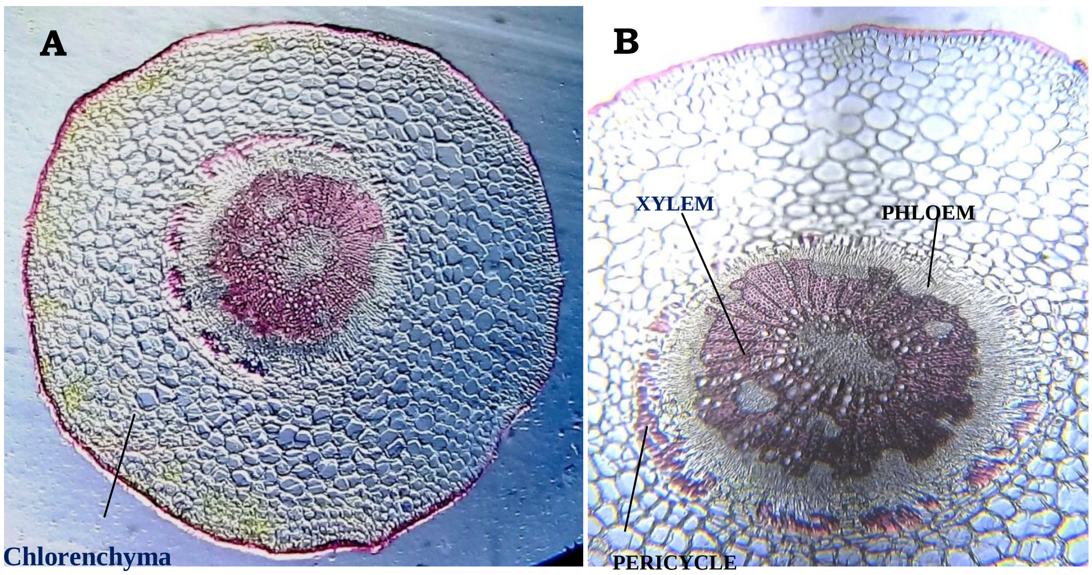


Fig. 6. A) TS of Petiole of *S. persica* Whole section. B) Enlarged view of the section. C) Midrib vascular bundle. The vessels are round of varying diameter.

Fig. 7. TS of *S. persica* Petiole – showing cortical parenchyma, endodermis and non-continuous sclernchymatous pericycle.

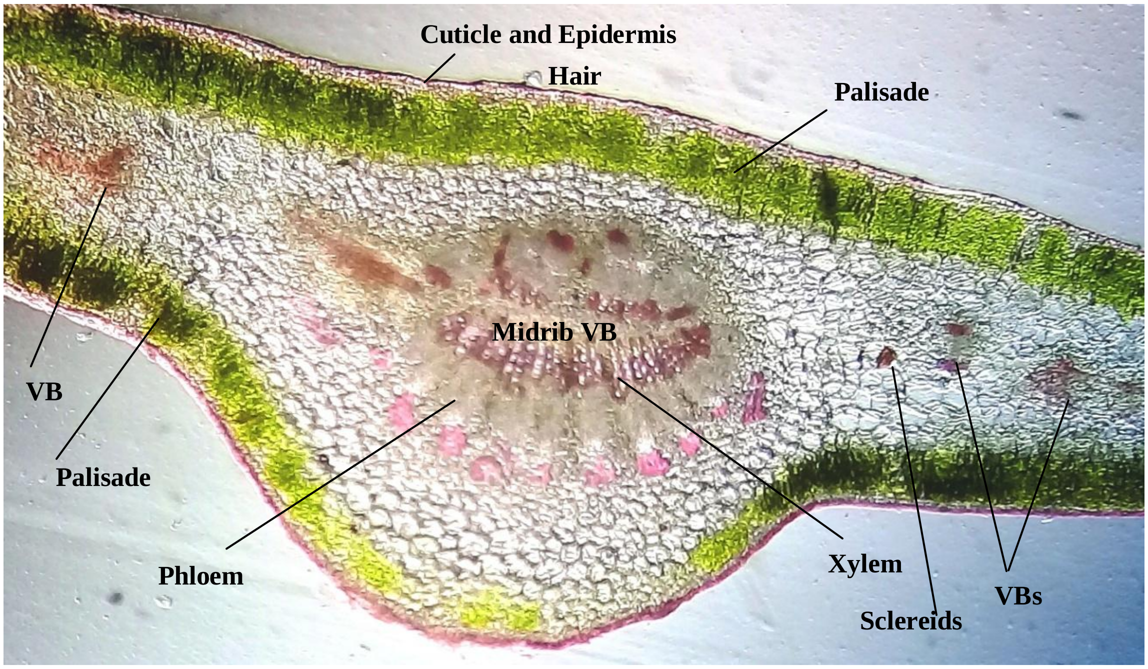


Fig. 8. TS of mature leaf of *S. persica* through the midrib region.

Fig. 9. TS of *S. persica* leaf - lateral margin of leaf showing mutiseriate epidermis on the corner and palisade on abaxial as well as adaxial surfaces (A) and a stoma on dorsal surface showing guard cells and a stomatal cavity (B).

SLA and SLM: SLA represents the resource-uptake efficiency and is highly correlated with leaf processes (Wright *et al.,* 2004). SLA of *S. persica* in a sample of 101 differentially aged leaves averaged to 56.69 ± 1.167 cm^2/g (corresponding to 5.67 ± 0.1167$m^2.kg^{-1}$. It varied from 37.60 to 97.27 (CV: 20.74%) that is 3.76 to 9.73 $m^2.kg^{-1}$. In 68.3% of the sample leaves SLA ranged from 51- 80cm^2 /g. This SLA value was somewhat low probably due to the deposition of salts in leaves. SLM on the other hand, averaged to 0.0182 ± 0.00035g/ cm^2 varying from 0.0103 to 0.0266 g / cm^2 (CV: 18.78%). SLA tended to distribute asymmetrically and SLM symmetrically. In around 79 % of the sample leaves, SLM ranged from 0.0151 to 0.0225g /cm^2. Under salinity SLA is reported to decrease in *S. persica* growing in Jazan region of Southeastern Saudi Arabia near Sandi/Yemeni border. Tounekti *et al.* (2017) reported decline in SLA of S. persica leaves from around 11$m^2.kg^{-1}$ DW in non-saline condition to 6.0 $m^2 kg^{-1}$ DW). Our SLA data appears to agree with Tounekti *et al.* (2017). Lower values of SLA are said to contribute long leaf life-span, nutrient retention and protection from dehydration of plants. It indicates more dry matter allocation to leaf which is much important in saline environment due to less availability of nutrients under salinity (Poorter and Garnier, 2007; Osnas *et al.,* 2013).

In *S. persica*, SLA was found to be non-significantly correlated linearly with LL (r = 0.089, p < 0.375), LB (r = **-** 0.187, p < 0.061) and LAM (r = -0.142, p < 0.156). Khan (2008) have also reported SLA to be non-significantly correlated linearly with leaf length, leaf Breadth and LAM but significantly correlated with leaf dry matter content and SLM, negatively. Curvilinear or logarithmic relationship of SLA with leaf area has also been reported to be better fit in *Nicotiana plumbaginifolia*.

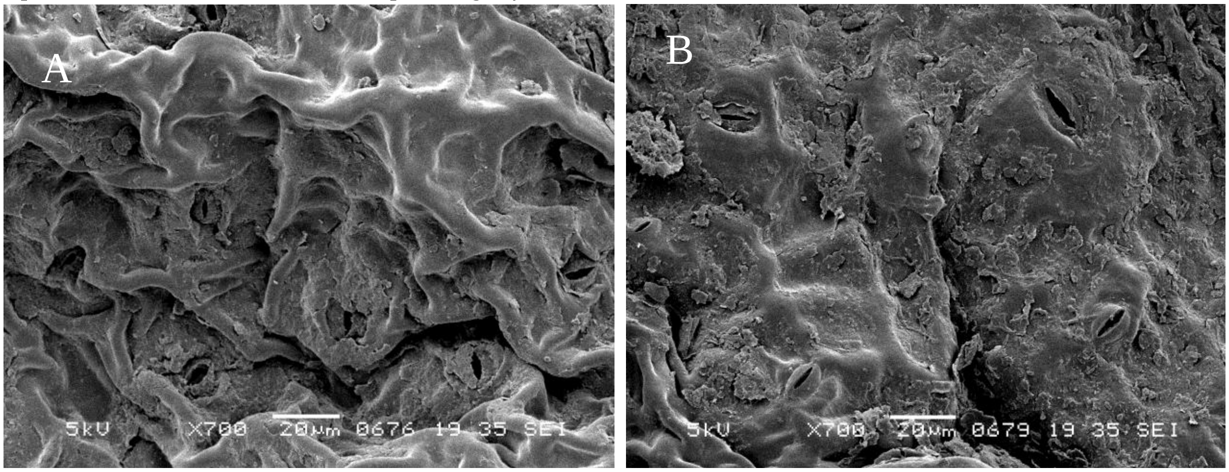


Fig. 10. Dorsal (A) and ventral surface (B) of leaf of *Salvadora persica*.

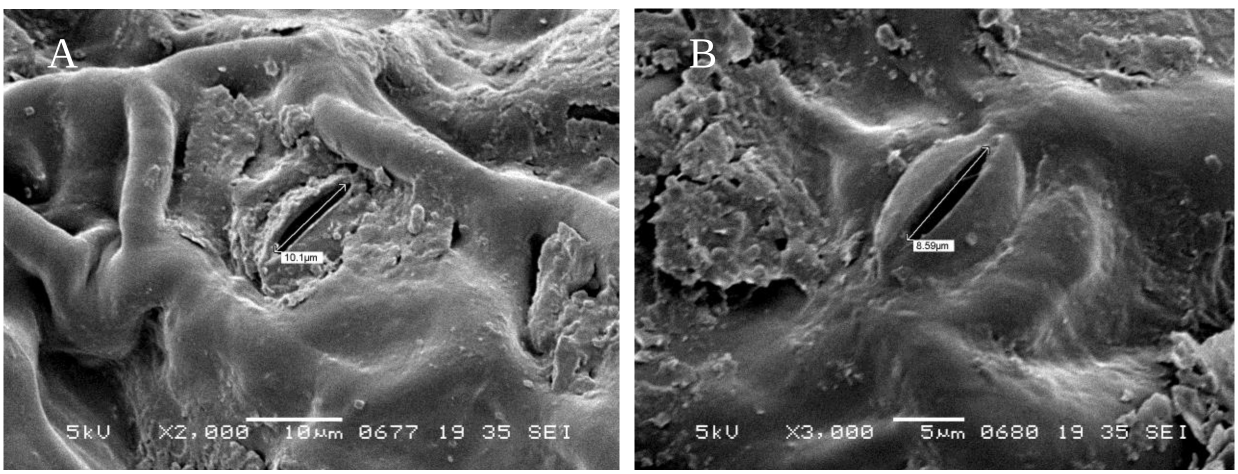


Fig. 11. Stomata on dorsal surface (A) and ventral surface (B) of leaf of *S. persica*. Stomatal ledge aperture size dorsal: 10.1 um in length; ventral 8.59 um. Several Flakes like waxy crystalloids may be seen.

SLA has been reported to vary with position of the leaf on the plants of *Nicotiana plumbaginifolia* (Khan, 2008). Among the four types of leaves recognizable in *Ficus religiosa* (red tender leaves, reddish green developing leaves, yellow green maturing leaves and dark green mature leaves) SLA, SLM, LDM, succulence and moisture content of leaf were found to be the plastic traits. Leaf area, leaf dry matter, LDM and SLM increased significantly with the growth and maturity of the leaves. Pink tender leaves had higher SLA and conversely low LDM and SLM. SLA was low in dark green mature leaves as compared to immature leaves. The tender leaves were more succulent than maturing and mature leaves (Khan, 2009). Liu *et al.* (2016) reported SLA to increase under shade. SLA is also reported to decrease with solar-irradiance in three sites both within and among species comparisons in tropical cloud forest of Bawangling Nature Reserve, South China (Long *et al.*, 2011). Ackerly *et al.* (2002) reported that at community level in Chaparral woody plants, the leaf size and SLA both declined with increasing insolation. However, leaf size and SLA were not correlated significantly across species suggesting that these two traits are decoupled and associated with different aspect of performance along the environmental gradient. Li *et al.* (2005) have reported variations in specific leaf area (SLA) and leaf dry matter content (LDM) in 20 annual and perennials species that showed different distributional patterns in the Kerqin Sandy Land in Northern China. In our studies with *S. persica*, SLA was found to be non-significantly correlated linearly with L, B and LAM but significantly correlated with leaf dry matter and SLM, negatively. Our results appear to agree with Wilson *et al.* (1999). They reported that SLA is quite variable between replicates and is influenced with leaf thickness. Leaf dry matter, in comparison to SLA, they opined to be a better parameter being independent of leaf thickness. Wright *et al.* (2004), however, opined that SLA is highly correlated with leaf process such as maximum photosynthetic rate. Under salinity, SLA is reported to decrease in *S. persica* (Tounekti *et al.* (2018). Lower values of SLA contribute long leaf lifespan, nutrient retention, and protection from dehydration. It indicates that in succulent leaves of *S. persica* more dry matter is allotted to leaf. This is important because nutrients are less available in saline soils (Poorter and Garnier, 2007; Osnas *et al.*, 2013).

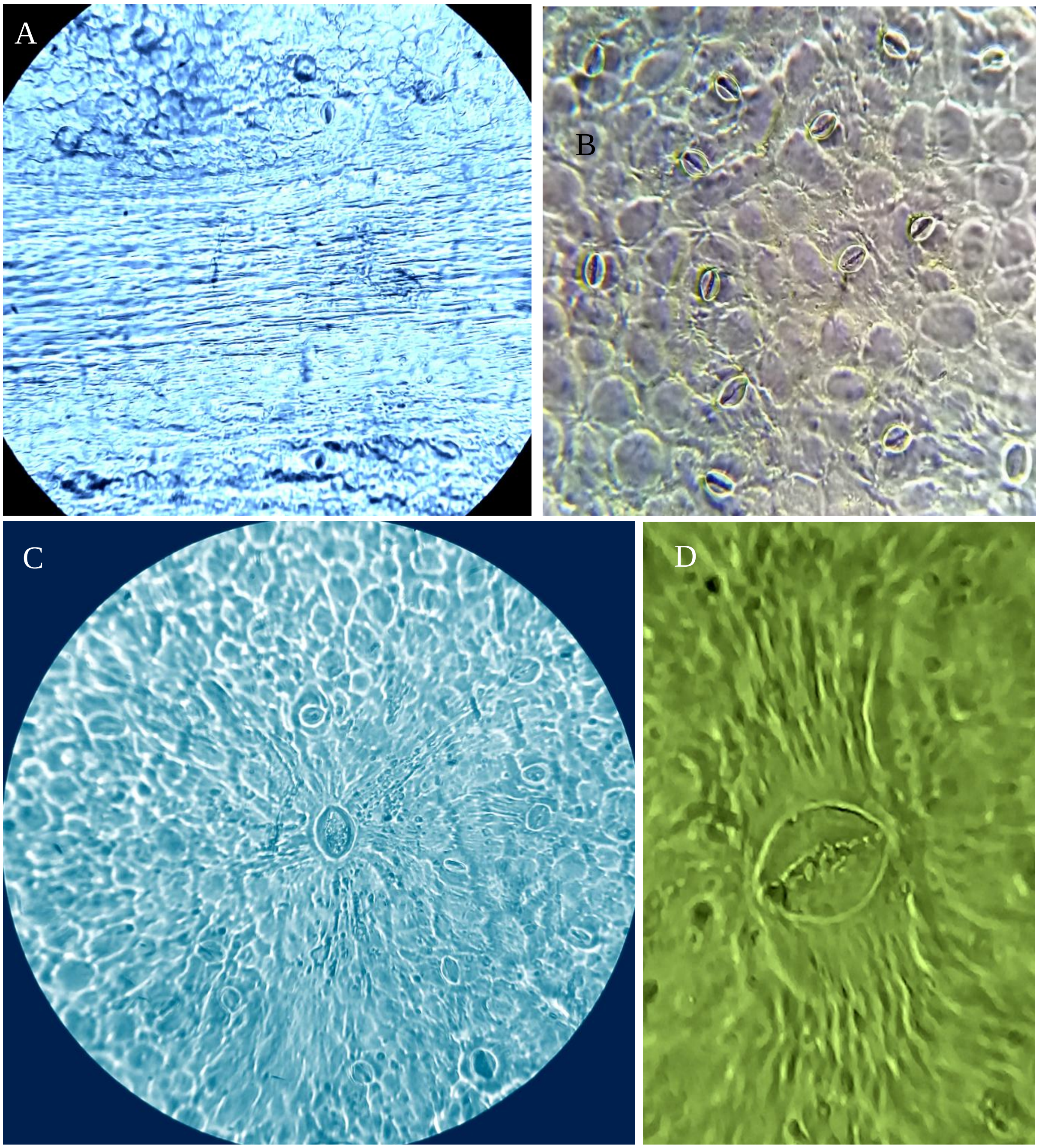


Fig. 12. The dorsal surface of leaf of *S. persica*. A, Surface over midrib region bears thick cuticle but devoid of stomata; B, Stomata (generally paracytic) distributed in a laminar Island – stomata in depressions; C, A large anomocytic stoma (*Sensu* Prabhakar, 2004) with fine radiating stiriations. The surrounding stomata are smaller, and D, An enlarged view of single stoma (paracytic) with cuticular strie forming wing-like structure over two neighbouring cells or subsidiaries. Magnification: A, B, & C (45 x10 X) and D (45 x10 X; Zoomed).

Surface micromorphology

Epidermal cells: The epidermal cells of leaf surface were irregular to polygonal in shape. They were cuticularized and papillose. The periclinal top surface plateau appear to be laid with cuticle first and deposition extending to the lateral wall (Fig. 13 and 14A). Cuticular encrustation appears to increase with age resulting masking of the epidermal surface structure to a great extent and showing only the guard cells of the stomata here and there protruded like eye-balls situated but in depression (Fig. 12D & 14B). Cuticle layer seems to be a sheet with some striations more prominent near stomata (Fig. 12 C and D). In scanning electron micrographs (Fig. 11), on magnification of 2000 and 3000X, on dorsal as well as ventral surface there occurred some flakes like crystalloids. Increase in the *in situ* salinity is reported to increase the amount of leaf epicuticular waxes in this species (Gururaja and Babu, 1997). These crystalloids were seen fusing into sheet and lumps and were probably provided thermal reflectance and water repellency to the lamina in the tidal environment.

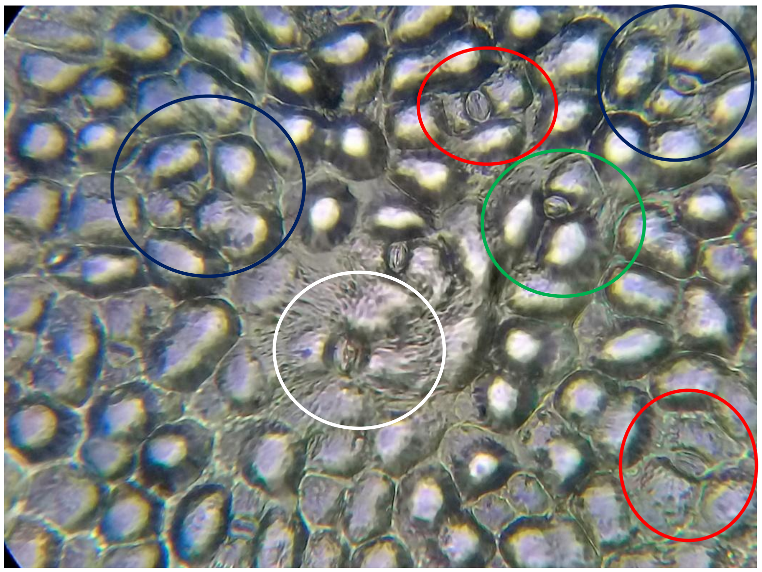


Fig. 13. Several types of stomata [paracytic (red circles), tetracytic (blue circle), anisocytic (green circle), staurocytic (white circle); Sensu Prabhakar, 2004] on dorsal surface of *S. persica* leaf. Note that the epidermal cells are papillose – conically raised above. Cuticular deposition appears to take place first at the top periclinal surface. Magnification: 45 x 10 X, zoomed).

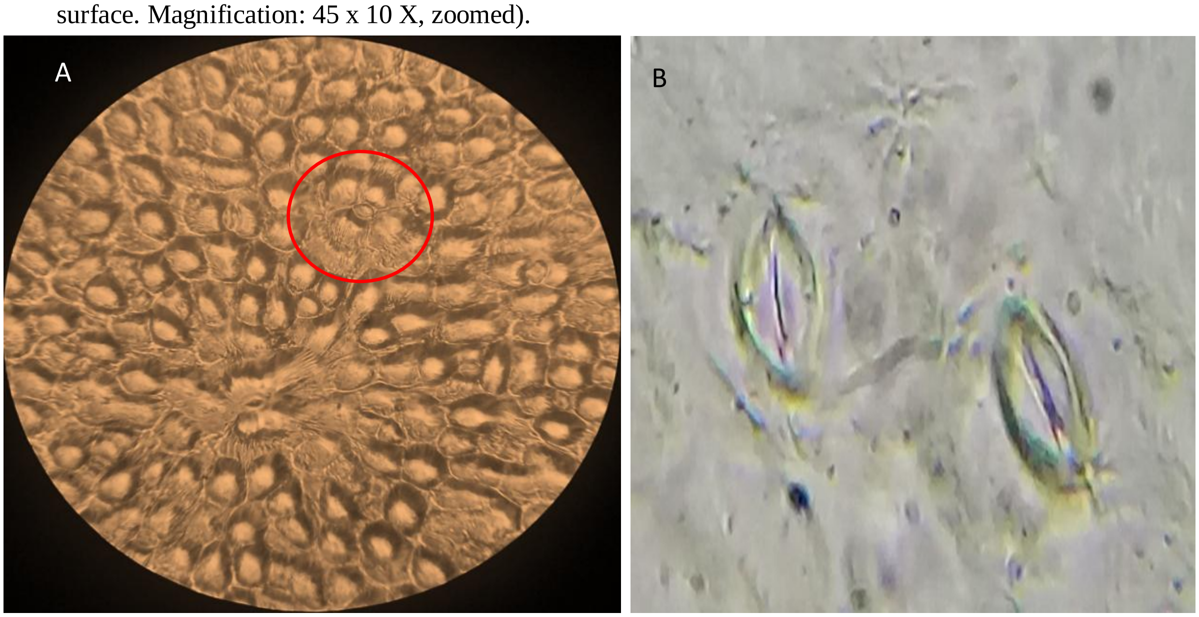


Fig. 14. Dorsal surface of leaf. A) A tetracytic stoma (Sensu Prabhakar, 2004) – two polar and two lateral surrounding cells. B) Thick cuticular sheet over the dorsal surface of the leaf masking the epidermal structural details. Magnification: A (45 x10 X) and B (45 x 15 X; zoomed).

Stomata: Leaves of *S. persica* are amphistomatic as also reported by Manavalan *et al.* (2010) in a sample from Madurai (India). Stomata were oval, and of several types present on both dorsal and ventral surfaces (Fig. 10-15). Stomata were oriented in various directions. They had guard cell with well-developed outer ledges arched like dome. *Sensu* Prabhakar (2004), the nail polish imprint of leaf dorsal and ventral surfaces exhibited paracytic, tetracytic, staurocytic, anisocytic and anomocytic type of stomata (Fig. 12 - 15). Watson and Dallwitz (1992) reported three types of stomata in *S. persica* – Anomocytic, paracytic or anisocytic. Inamdar (1969) has reported a diversity of stomata on *S. persica* leaves – paracytic, anisocytic, anomocytic (of perigenous origin), and some abnormal ones such as stoma with single guard cell, stomata with unequal guard cells, contiguous stomata, or clustered ones. Metcalfe and Chalk (1979) reported two types of stomata (paracytic and anomocytic) in Family Salvadoraceae. Karejo *et al.* (2010) described anomocytic stomata *in S. persica* from Jamshoro, Sindh, Pakistan

In our studies, as determined in three different mature leaves of comparable size, the stomatal density varied substantially averaging to 218.14 ± 3.64 stomata per mm^2 on dorsal surface and 241.99 ± 3.06 stomata per mm^2 on ventral surface (Table 5). Karejo *et al.* (2010) described more stomata on dorsal side of *S. persica* leaf. *S. persica*, in our studies, had greater stomatal density on ventral surface which is said to be common in species occurring in xeromorphic environments (Cutter, 1986).

On younger leaf stomatal density was higher (389.23 ± 6.29 dorsally and 328.09 ± 6.49 ventrally). It is known since Salisbury (1928) that stomatal density is related to leaf size inversely. Stomatal density on young and small terminal leaflets of *C. fistula* was reported to be quite higher than that on relatively larger leaflets (Khan and Zaki, 2019). Peel *et al.* (2017) also reported that stomatal density is inversely related to leaf area ($r = -0.29$, $p < 0.001$), especially leaf width ($r = -0.31$, $p < 0.001$). The decrease in SD in larger leaves, as compared to the smaller ones, may be attributed to the foliar epidermal cells' expansion. Young leaves have large number of stomata but as leaf expands the density declines (Gay and Hurd, 1975). Also, lower radiation is known to affect stomatal densities (Rawson and Craven (1975).

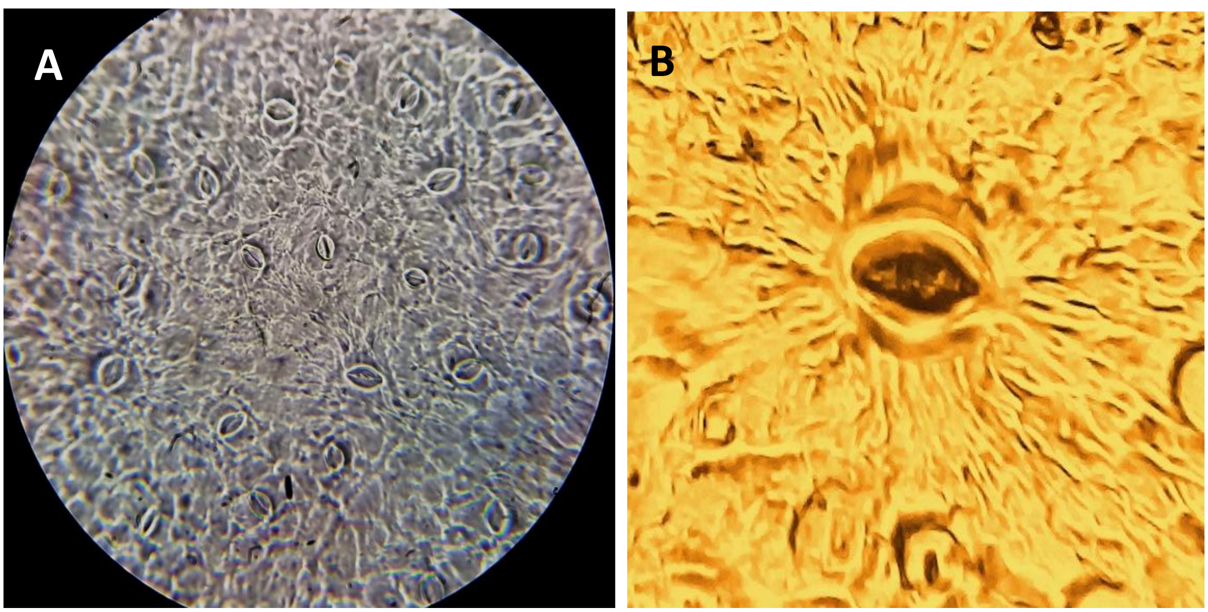


Fig. 15. Ventral surface of leaf of *S. persica.* A) Some paracytic and anomocytic stomata may be seen. B) A large anomocytic stoma with apparent cuticular striations. Magnification: A (45x10 X) and B (45 x 10 X; Zoomed).

Under desert conditions, *Salvadora persica* leaves are reported to contain 69 stomata per mm^2 and a stoma measuring around 22µm (Stocker. 1971). Under saline-arid conditions stomata appear to reduce in size. Some studies suggested that stomatal density in *S. persica* is up to 50% higher on the upper surface (Brown and Miles, 2012). Gibson (2012), however, reported similar stomatal density on abaxial and adaxial surfaces of *S. persica* leaves. Our results are not in agreement with Gibson (2012) or Stocker (1971). We found much higher stomatal density on both surfaces of the leaf of this species when growing in the fringes of the supra-littoral zone of Karachi coast often inundated with tidal Seawater (Table 5). Contrary to it, Tounekti *et al.* (2018) reported quite low foliar stomatal density (49 ± 3 adaxially and 61 ± 8 abaxially) in *S. persica* from non-saline sites of Jazen province from Saudi Arabia which decreased in saline sites. In our case, stomata were smaller (16.3 – 17.01 µm in length and 10.7 – 11.9 µm in width; Table 6) than that reported by Stocker (1971). Stomata in *S. persica* leaves on an average were equal in size on dorsal (16.32 x 10.71 µm and ventral surface (17.01 x 11.90 µm) as measured in nail polish imprint of the leaves (Table 6). The pore of the stomatal ledges measured in scanning electron micrographs was found to be 8.59 to 10.1 µm (Fig. 11 A and B; SEM). Stomatal size is reported to decrease under severe water stress in quadratic parabolic manner in a grass (*Leymus chinensis*) (Xu and Zhou, 2008).

Leaf area and stomatal density play an important role in maintaining water balance, and gas exchange is regulated by stomatal aperture and density, the two traits that vary intraspecifically in response to environmental conditions, such as water stress and salinity. Peel *et al*. (2017) evaluated the effects of salinity on stomatal density, leaf area and plant size in *R. mangle*. The larger leaves from the low salinity site had lower densities of stomata (65.0 stomata.mm^{-2}, SD = 12.3) and increasing salinities did not decrease stomatal density (intermediate salinity site: 73.4 stomata.mm^{-2}, SD = 13.5; high salinity site: 74.8 stomata.mm^{-2}, SD = 17.3). Salinity may increase stomatal density by causing reduction of leaf size (Peel *et al*., 2017). Salinity reduces the stomatal density in *Kandelia candel* seedlings (Qiu *et al*., 2007). As stomatal size decreases with salinity, there must be increase in stomatal density optimizing gas control to meet desired photosynthetic capacity (Franks and Farquhar (2007). Xu and Zhou (2008) have reported positive effect of moderate water stress on stomatal density in a grass (*Leymus chinensis*). Gill and Dutt (1962) reported varying results of salinity effects on stomatal density in various cultivars of *Hordeum vulgare* which increased with salinity in various cultivars but stomatal size decreased. De Villiers *et al.* (1996) reported salinity to significantly increase stomatal density in leaf of *Atriplex semibaccata* – adaxially from 75.63 (control) to 100.69 (2/3 Seawater salinity). Carbon dioxide concentration and temperature may influence the stomatal density on the leaf (Beerling and Chaloner, 1993). Warming may significantly decrease the average nearest neighbour distance between stomata (Zheng *et al.* (2013). As structure, development and patterning of stomata on the leaf surface is the function of complex processes, they should be viewed from evolutionary, physiological, ecological and organ view-point (Croxidale, 2000).

Table 5. Stomatal density per mm^2 in freshly collected mature and young leaves of *S. persica* (Dorsal surface).

Parameter	Leaf I (Mature)	Leaf II (Mature)	Leaf III (Mature)	Pooled data (Mature)	Young Leaf*
Dorsal surface					
N	50	50	50	150	50
Mean	269.90	186.95	197.56	218.14	389.23
SE	4.187	2.978	3.396	3.644	6.286
CV (%)	10.97	11.27	12.55	20.46	11.42
Minimum	206.41	137.61	137.61	137.61	304.70
Maximum	353.84	235.90	245.72	353.84	501.28
Ventral surface					
N	50	50	50	150	50
Mean	275.015	237.861	213.09	241.99	328.09
SE	3.9664	4.1178	3.6012	3.0579	6.4888
CV (%)	10.20	12.24	11.95	15.48	13.99
Minimum	226.07	157.26	147.43	147.43	235.9
Maximum	324.36	294.87	275.21	324.36	432.47

Table 6. Stomatal length and width (µm) on dorsal and ventral surface of *S. persica* leaf inclusive of some large stomata.

Parameters	Dorsal surface		Ventral surface	
	Stomatal length	Stomatal width	Stomatal length	Stomatal width
N	55	55	55	55
Mean	16.32	10.71	17.01	11.91
SE	0.5092	0.2917	0.4717	0.2844
CV (%)	23.14	20.21	20.57	17.71
Minimum	8.00	6.40	9.68	8.00
Maximum	22.40	14.40	25.60	20.80

High stomatal density seen in *S. persica* in our studies could be due to diversification in the genus *Salvadora* and its species namely *S. persica* owing to genetic and environmental reasons. Verdcourt (1964) had suggested much variation in *S. persica*. Tahir *et al.* (2010) have reported a new species, *Salvadora alii* from Jamshoro, Sindh – a species with ovate leaf, white fruits and seeds globular with large number of prominent pits. Later, it was described from Madhya Pradesh, India (Mujaffar *et al.,* 2012). Our sample plant bearing red fruits appeared to be *Salvadora persica*. Karejo *et al.* (2010) reported that both branch and seed features presented remarkable subspecies variations in morphology and biochemical markers (seed protein) within *Salvadora persica* and indicated a possibility of occurrence of two subspecies or varieties in *S. persica*, in Sindh, Pakistan. The presence of two varieties (i.e. *S. persica* L. var. *indica* Wight and *S. persica* var. *tuticornica*) has been reported from India (Rao and Chakraborti, 1996). None of these taxa have, however, been studied micromorphologically in detail.

Our local halo-physiotypic flora in general and *S. persica* in particular need to be investigated from diverse habitats of their occurrence with respect to their variation in leaf micromorphology especially in coastal and inland halo-xeric environments of Pakistan. There appears a possibility of occurring stomatal density mutants in *S. persica*. Such mutants have been reported in *Arabidopsis thaliana* presenting several-fold variation in stomatal density (Doheny-Adams *et al.,* 2012; Lawson *et al.*, 2014).

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