

TAXONOMICAL INVESTIGATION IN THE LEAVES OF SOME SALSOLA L. SPECIES IN THE MEDITERRANEAN COASTAL STRIP



Original Research Article

ISSN : 2456-1045 (Online)

(ICV- BIOS /Impact Value): 63.78

(GIF) Impact Factor: 4.126

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Journal Code: ARJMD/BIOS/V-22.0/I-1/C-4/FEB-2018

Category : BIOLOGICAL SCIENCE

Volume : 22.0 / Chapter- IV / Issue -1 (FEB-2018)

Journal Website: www.journalresearchijf.com

Paper Received: 07.03.2018

Paper Accepted: 12.03.2018

Date of Publication: 16-03-2018

Page: 19-26



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Citation of the Article

Taia W.K ; Shela M.A ; Kogali A.A ; Almaged A.M.A (2018) Taxonomical Investigation in the leaves of some Salsola L. species in The Mediterranean coastal strip.; Advance Research Journal of Multidisciplinary Discoveries.22.0,C-4 (2018) 19-26

ABSTRACT

Leaf macro- and micro characters in six Salsola species in Egypt grown in the Mediterranean coastal strip from Alexandria to El-Dabaa district. The work based on both light and Scanning Electron Microscopes (SEM). The studied species are *S.kali*, *S.longifolia*, *S.tetrandra*, *S.tetragona*, *S.volkensii* and *S.inermis*. The results obtained showed significant variations in the leaf macro-characters within the studied species. Epidermal cells and trichome characters are greatly varied within the studied taxa. Statistical analyses evaluated the differences between the taxa. The result of ANOVA test revealed that both the stem width and leaf length are highly significant between the studied taxa while the stem length is significantly different and leaf width is insignificant. The resulted dendogram separate *S.kali* at dissimilarity distance of 0.54 and *S.longifolia* at dissimilarity matrix of 0.43 and the other four species clustered together. This group of the four species separate *S.volkensii* at dissimilarity matrix of 0.25 and *S.inermis* at 0.24 and still *S.tetragona* and *S.tetrandra* very closely related. This result coordinates with the leaf morphological characters as both *S.kali* and *S. longifolia* can be easily distinguished from the other four species. Identification key for the studied *Salsola* species has been done. . This work considers guide in the identification and circumscription of the studied species.

Keywords : Epidermal cells-Leaf morphology- *Salsola* species- Stomata- Taxonomy-Trichomes.

I. INTRODUCTION

Genus *Salsola* is a cosmopolitan group of plants which distributed and naturalized all over the world. Species belong to this genus found in most arid places of the world, along sea shores, in grassland, wastelands, roadsides and desert areas (Krzaczek *et al.*, 2009). Plants belonging to this genus tolerate with dryness, pH fluctuation and high degree of salinity. The genus is considered one of the very difficult genera from the taxonomic point of view for the great resemblance in the morphological features between its species (Turki, 1999). *Salsola* has a controversial subgeneric classification, and its monophyly has been questioned, as has the recognition of such genera as *Climacoptera* (Botschantzev 1956; 1969b; Prato 1986), *Halothamnus* (Iljin 1936; Botschantzev 1981b), *Darniella* (Brullo 1984), *Fadenia* (Aellen and Townsend 1972), and *Xylosalsola*, *Nitrosalsola*, and *Newcaspsia* (Tzvelev 1993). The detailed revision of most species groups of the genus *Salsola* was carried out by Botschantzev (1989). Based on the earlier works of Fenzl (1851), Ulbrich (1934) and Iljin (1936 a), as well as on morphological features of vegetative organs Botschantzev, recognized six systematic sections of *Salsola*. The taxonomic system of genus *Salsola* worked by Botschantzev (1989) is still widely accepted and in the present study we also use it as a base:

Section *Caroxylon* (Thunb.) Fenzl.
Section *Malpigila* Botsch.
Section *Cardiandra* Aellen
Section *Belanthera* Iljin
Section *Coccosalsola* ,subsec.. *Arbuscula* (Fenzl.)
Section *Salsola*, subsec. *Kali* (Ulbrich)

In Egypt the genus comprise twelve species distributed in the Mediterranean coastal region, Sinai, Arabic desert and Gebel Elba (Täckholm, 1974; Boulos, 1999). Only six of them are found in the Mediterranean coastal region, west of Alexandria and subjected in this study. The aims of the study are to investigate how much vegetative characters can affect the delimitation of the taxa and can help in the identification of the species, to determine whether they can reinforce the specific boundaries and clarify the evolutionary line between the taxa or not. The study based on Stereo, light (LM) and scanning electron microscopes (SEM), as well as on vegetative examinations.

II. MATERIALS AND METHODS

Field trips were carried by both Dr. Sheha and Abdel Maged during the period from April 2012 to October 2014. Fresh materials of six *Salsola* species were collected from the Mediterranean coastal regions, west Alexandria to Eldabaa, Alexandria Egypt. Herbarium sheets were allocated in the Herbarium of Alexandria University, Faculty of Science (Table 1). Ten specimens from each species were subjected to morphological investigations and measurements.

For LM studies, the fourth leaf, from three individuals in each species, was washed with water to remove the dust and photographed using Stereomicroscope. Parts of the leaves were put in test tubes with 5ml. dist. water, 2 drop tepol, and 2 drop 1N HCl and warmed gently to remove the epidermal layer for light microscope study (LM).

For SEM studies, dried mature leaves were placed on stubs, coated with a thin layer of gold then scanned and photographed at 15 KV with JEOL JSM 5300 SEM at the Electron Microscope Unit, Alexandria University. The descriptive terms used for epidermal cells, stomata in this works follow Barthlott (1981), and that for hairs (trichomes) adopted after Al-Shammary and Gornal (1994).

All measurable characters were subjected to ANOVA test to qualify its significance in the circumscription of the studied

to show the relation between them. Clustering analyses of the resulted data was done using Past 3 (1999-2013).

Table 1. Collection data of the studied *Salsola* species

No.	Species	Localities and Date
1	<i>Salsola kali</i> L.	Burg El-Arab, 43 Km Alex Matrouh road 16.10.2012
2	<i>Salsola longifolia</i> Forssk.	80 Km west of Alex; Alex-Matrouh road sides (Omayed) 25.4.2013
3	<i>Salsola tetragona</i> Delile, Descr.	Burg El-Arab airport road 27.4.2014
4	<i>Salsola tetrandra</i> Forssk.	El-Hammam road, Mariout lake sides 16.10.2012
5	<i>Salsola volkensii</i> Schweinf. & Asch.	El-Omayed road, El-Khashm hill 20.11.2013
6	<i>Salsola inermis</i> Forssk.	80 Km west of Alex; Alex-Matrouh road sides 16.10.2012

III. RESULTS

Members of *Salsola* are Shrub, subshrubs, annual or perennial herbs, sometimes biennial in *S.tetragona*. Species of the genus found in arid, saline places and desert communities. They tolerate aggressive climate and dryness. Individuals of *S.volkensii* have decay fish smell. The stem macro-morphological characters are summarized in table (2). The stem has different shades of green color, except in *S.longifolia* L. the stem was pinkish green. They are glabrous, woody, and erect. The outline of the stem in T.S. is circular in most of the studied species, except in *S. kali* it is tetragonal and *S.longifolia* it is elliptic. The length of the stem ranges from 15 to 20 cm in *S.volkensii*, 20 to 25cm in *S.kali* and *S.inermis*, 25 to 35 cm in *S.tetrandra*, *S.tetragona* and *S.longifolia*. The width of the stem is very thin, mostly less than 1 mm. in all the studied species, except *S. kali* it range from 3 to 4 mm.

The leaf macro-morphological characters are summarized in table (2) leaves in most of the studied species are opposite decussate; *S.longifolia*, *S.tetragona* and *S.tetrandra*; alternate in *S.kali*. The leaves in *S.volkensii* and *S.inermis* both alternate and opposite decussate leaves can be found. The leaves narrow, simple, succulent and sessile, with different sizes, shapes and grades of green. (Table 2, plate 1). Leaf shape mostly orbicular, lanceolate in *S. kali*, tubular in *S.longifolia* and broadly ovate in *S. volkensii*. All studied species exhibit acute leaf apex except in *S.longifolia* which has rounded leaf apex and *S.kali* which has piliferous apex (Stearn, 1983). The leaf surface of all studied species possesses different types of trichomes except *S.longifolia* has glabrous surfaces (table 2 & plate 1).

Leaf microstructures

Using SEM leaf microstructures were studied and the results obtained are summarized in table (3). The studied species exhibit homogenous pattern of epidermal cells which was isodiametric straight except in *S.kali* has two type of epidermal cell pattern, the cells on the leaf margins were elongated in one direction while the cells around the midrib were isodiametric straight. Six surface patterns were recognized, scalariform pattern which found only in the internal cells of *S.kali* while irregular reticulate pattern in the external part of the epidermal cells, reticulate areolate pattern in *S.longifolia*, areolate pattern in *S.tetragona*, alveolate cellular arrangement *S.tetrandra*, and finally the ruminant pattern in *S.inermis* and *S.volkensii*. The cell shape in all the studied species was hexagonal, except in *S.kali* tetragonal or pentagonal. The wall of the epidermal cells varies in thickness within the different species and this variation can be due to environmental stresses as mentioned by Esau (1960). In this investigation *S.inermis* and *S.tetrandra* have no cutinized thin cell wall, while in the rest of the species the cell wall was

cutinized (**Plate 2**). The anticlinal walls straight in all studied species with channeled cell boundary except in *S.kali* it has raised cell boundary. The curvature of periclinal walls slightly flat in *S.kali* and convex in the rest of studied species, their texture granulate in *S.kali* and ornamented reticulate in the rest of studied species. The wax deposition takes different shapes and density in the studied species as illustrated in **table 3**; flax in *S.longifolia*, *S.tetrandra* and *S.volkensii*, granulate in *S.kali*, *S.tetragona* and *S.inermis*. Sparse in *S.kali* and *S.tetrandra*, moderate in *S.longifolia*, *S.tetrandra* and *S.inermis* and dense in *S.volkensii*. The stomata in all the studied species was kidney with two subsidiary cells of the paracytic type in *S.kali*, *S.longifolia*, *S.tetrandra* and anisocytic in *S.tetragona*, *S.volkensii*, and *S.inermis* (**Table 3, plate 2**).

The leaf surfaces are either glabrous in *S.longifolia* or enriched by unicellular or multicellular- uniseriate pointed hairs (**table 4**). The density of trichomes varied on the surface of leaves in *S.kali*. Dense trichomes in *S.volkensii* and woolly in *S.tetragona*, *S.tetrandra* and *S.inermis*. Trichomes were monomorphic in all the studied species except in *S.volkensii* it was dimorphic i.e. the leaves are covered by two types of hairs (**Plate 3**). Hair length and width were not measured, but relatively grouped in three categories as follows: short in *S.kali* species, long in *S.tetragona* and *S.tetrandra*, very long in *S.inermis*, two types of hairs noticed in *S.volkensii* one of them very long and the other type short (**Plate 3**). The hair width relatively wide in *S.kali*, moderate in *S.tetragona*, thin in *S.tetrandra* and *S.volkensii* and very thin in *S.inermis*. Hair apex was acute in all the studied species. The hair wall either smooth in *S.kali* or echinate in the rest of the hairy species (**Table 4 & Plate 3**). Three types of basal cell were recognized; normal epidermal cells in *S.tetragona* and *S.tetrandra*, the rosette-like epidermal cells in *S.inermis* and in the long hairs of *S.volkensii*, rosette papillate in the short hairs of *S.volkensii* (**Plate3**). *S.kali* has no basal cells as the hairs are protruded directly from the epidermal cells (**Plate 3**).

The result of ANOVA test revealed that both the stem width and leaf length are highly significant between the studied taxa, while the stem length is significantly different and leaf width is insignificant (**table 5**). The resulted dendrogram separate *S.kali* at dissimilarity distance of 0.54 and *S.longifolia* at dissimilarity matrix of 0.43 and the other four species clustered together. This group of the four species separate *S.volkensii* at dissimilarity matrix of 0.25 and *S.inermis* at 0.24 and still *S.tetragona* and *S.tetrandra* very closely related. This result coordinates with the leaf morphological characters as both *S.kali* and *S.longifolia* can be easily distinguished from the other four species.

Table 2: Vegetative macro-morphological characters in the studied *Salsola* L. species
 Key to table 2, Smell += light, += moderate, +++= fishy smell

Sl. No.	Characters	<i>S.kali</i>	<i>S.longifolia</i>	<i>S.tetragona</i>	<i>S.tetrandra</i>	<i>S.volkensii</i>	<i>S.inermis</i>
	Habit	Perennial	Annual	Biennial	Annual	Annual	Perennial
1	Stem Smell	+	+	++	++	+++	+
2	Colour	Green	Pinkish green	Pale green	Pale green	Pale green	Green
3	Shape	Tetragonal	Elliptic	Circular	Circular	Circular	Circular
4	Length(cm)	20 – 25 (22.7±2.5)	23 – 32 (26.7±4.7)	25 – 35 (29.3 ± 5.1)	25 – 30 27.7±2.5)	15 – 20 (17.7±2.5)	22 – 25 (23.67±1.5)
5	Width(mm)	3 – 4 (3.56±0.43)	0.6 – 0.8 (0.7±0.08)	0.8 – 1.1 (0.95±0.13)	0.5 – 0.8 (0.65±0.13)	0.4 – 0.6 (0.5±0.08)	0.8 – 1.1 (0.95±0.13)
6	Leaf Arrangement	Alternate	Opposite Decussate	Opposite Decussate	Opposite Decussate	Opposite-Alternate	Opposite-Alternate
7	Colour	Green	Green	Pale green	Pale green	Pale green	Green
8	Length(cm)	(1- 2 1.5±0.5)	1.5 – 2 (1.7±0.3)	0.2 – 0.3 (0.23±0.06)	(0.2 – 0.2 0.2)	0.4 – 0.7 (0.53±0.15)	0.1 – 0.2 (0.17±0.05)
9	Width(cm)	0.2 - 0.3 (0.27±0.05)	0.2 – 0.3 (0.23±0.06)	0.2 – 0.3 (0.27±0.06)	(0.2 – 0.2 0.2)	0.15 – 0.25 (0.2±0.05)	(0.2 – 0.2 0.2)
10	L/W	5.56	7.39	0.85	1	2.65	0.85
11	Shape	Lanceolate.	Tubular	Orbicular	Orbicular	Broadly.Ovate	Orbicular
12	Apex	Piliferus	pointed	Acute	Acute	Acute	Acute
13	Surface	Hairy	Glabrous	Hairy	Hairy	Hairy	Hairy

Table 3 Vegetative micro-morphological characters in the studied *Salsola* L. species

Key to table 3: Characters, Sym.Epi.Cells= Symmetry of epidermal cells, SI= Stomatal Index, ST= Stomatal type, Para.= Paracytic, Aniso.= Anisocytic.

Taxa→ Characters↓	<i>S.kali</i>	<i>S.longifolia</i>	<i>S.tetragona</i>	<i>S.tetrandra</i>	<i>S.volkensii</i>	<i>S.inermis</i>
Sym. Epi. Cells	Heterogeneous	Homogeneous	Homogeneous	Homogeneous	Homogeneous	Homogeneous
Cellular arrang.	Scalariform – irreg.reticulate	Reticulate-areolate	Areolate	Alveolate	Ruminate	Ruminate
Outline of cells	elongated / isodiametric	isodiametric straight	isodiametric straight	isodiametric straight	isodiametric straight	isodiametric straight
Cell shape	Pentagonal or tetragonal	Hexagonal	Hexagonal	Hexagonal	Hexagonal	Hexagonal
Wall thick.	Thick	Thick	Thick	Thin	Thick	Thin
Anticlinal wall	Raised	Channeled	Channeled	Channeled	Channeled	Channeled
periclinal wall	Flat	Convex	Convex	Convex	Convex	Convex
Wax deposition Type	Granules	Flax	Granules	Flax	Flax	Granules
Density	+	++	+	++	+++	++
Stomata						
SI.	11.62	8.89	10.48	7.3	11.8	14.47
ST.	Para.	Para.	Aniso.	Para.	Aniso.	Aniso.

Table 4 Trichome characters

Key to table 4: Density ++= hairy, +++= Densely hairy, ++++= Woolly V.long= very long, Mod.=Moderate, Orn.= Ornamentation, Unicell.= Unicellular, Mulcell.= Multicellular.

Taxa→ Characters↓	<i>S.kali</i>	<i>S.tetragona</i>	<i>S.tetrandra</i>	<i>S.volkensii</i>		<i>S.inermis</i>
Density	++	++++	++++	+++		++++
Symmetry	Mono-morphic	Mono-morphic	Mono-morphic	di-morphic		Mono-morphic
Length	Short	Long	Long	Short	V.long	V.long
Width	Wide	Mod.	Thin	Thin	Thin	V.thin
Wall Orn.	Smooth	Echinate	Echinate	Echinate	Echinate	Echinate
Echinae Density	-	++	++	++	++	+++
Type	Unicell.	Mulcell.	Mul cell.	Unicell.	Mulcell.	Mulcell.
Basal cell	Absent	Normal	Normal	Rosette papillate	Rosette	Rosette

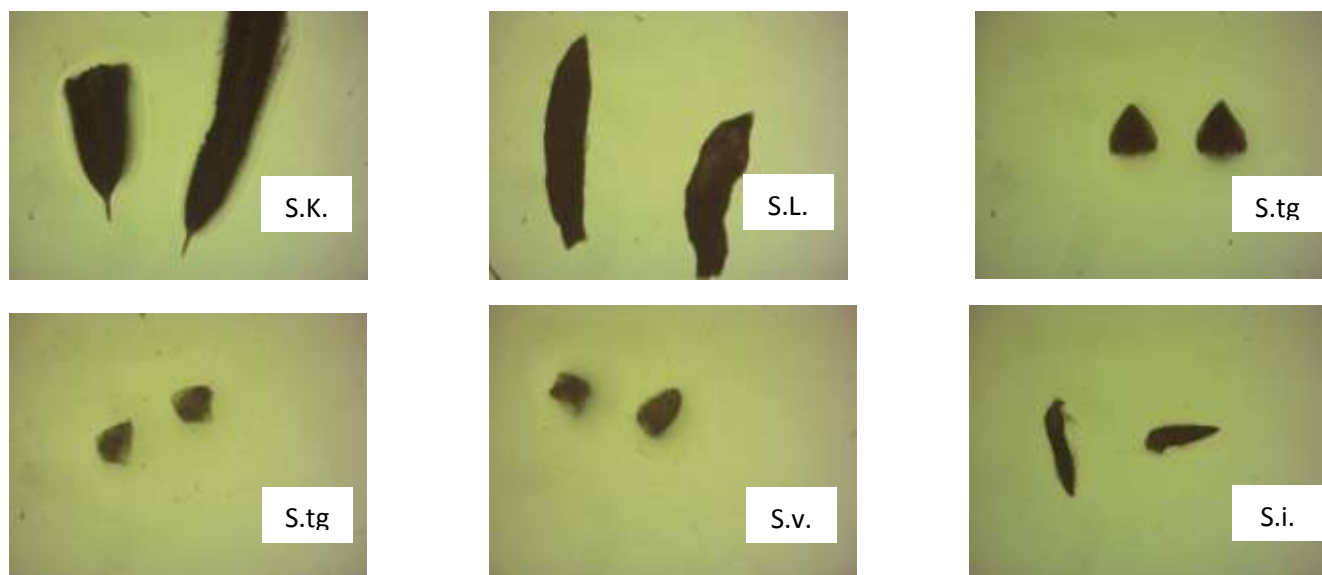


Plate 1 Leaf shapes in the studied species; S.K.= *Salsola kali*, S.L.= *Salsola longifolia*, S.tg.= *Salsola tetragona*, S.td.= *Salsola tetrandra*, S.v.= *Salsola volkensii*, S.i.= *Salsola inermis*, — = 1mm.

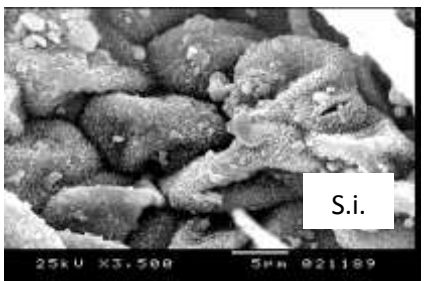
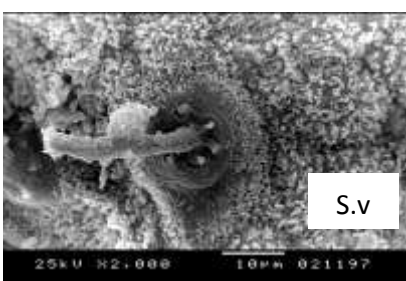
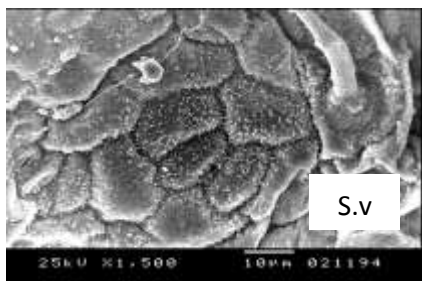
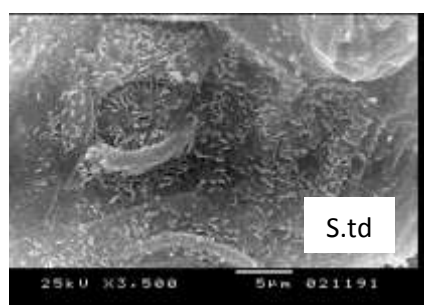
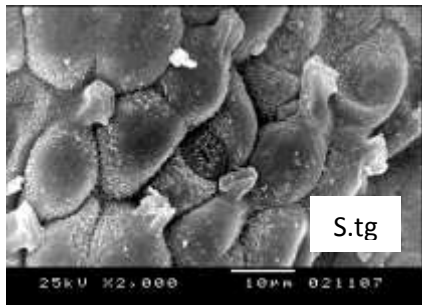
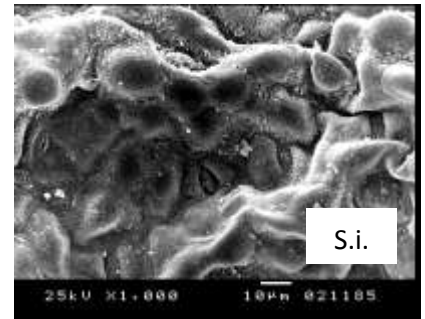
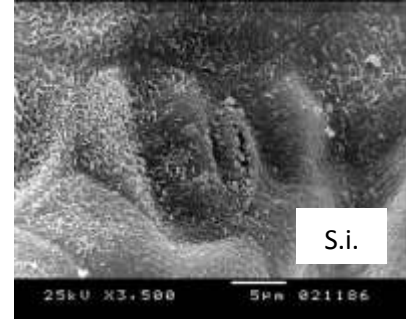
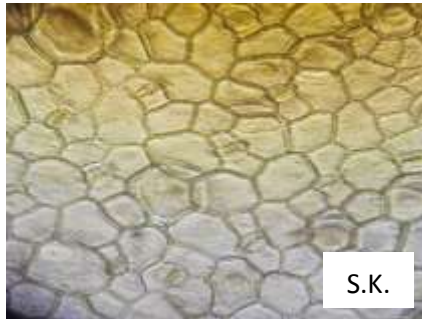
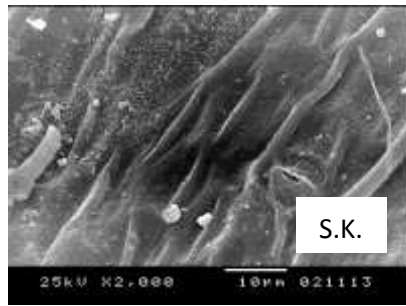
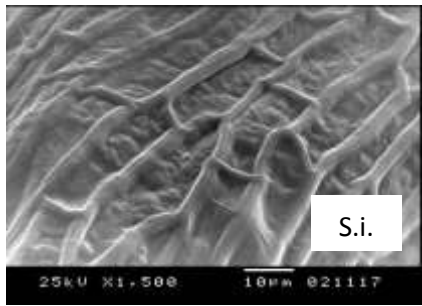


Plate 2 Leaf epidermal cell characters by both light and SEM ___ = 100 µm (LM)

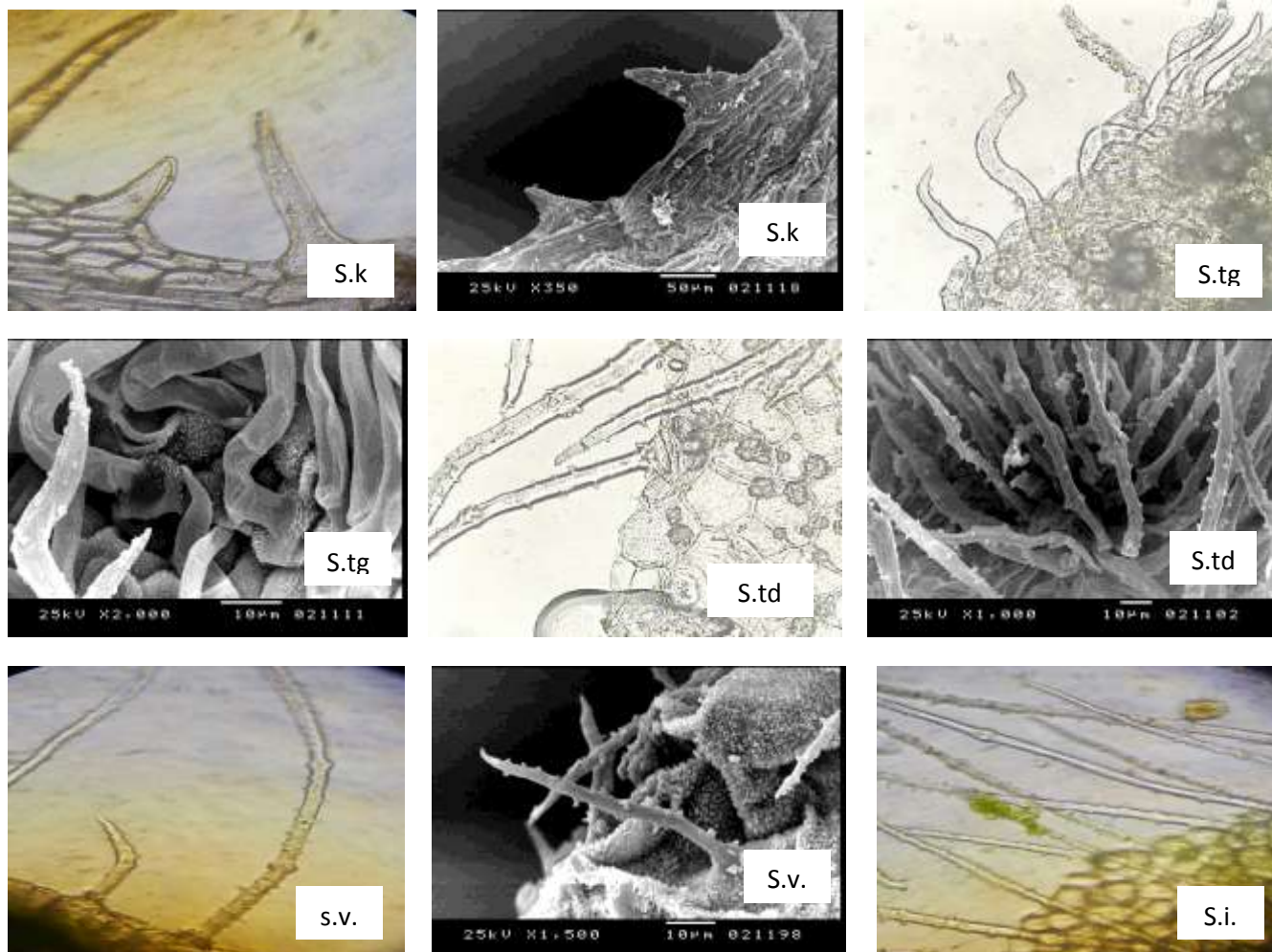


Plate 3 Trichome characters by both light and SEMs $__ = 100 \mu\text{m}$ (LM)

Table 5: Macro-Morphological Characters Subjected To ANOVA Test

	F value	F critical	P value
Stem length	4.564762	3.105875	0.014541*
Stem width	128.0621	2.772853	2.03E-13**
Leaf length	23.62182	3.105875	7.98E-06**
Leaf width	1.546667	3.105875	0.247848

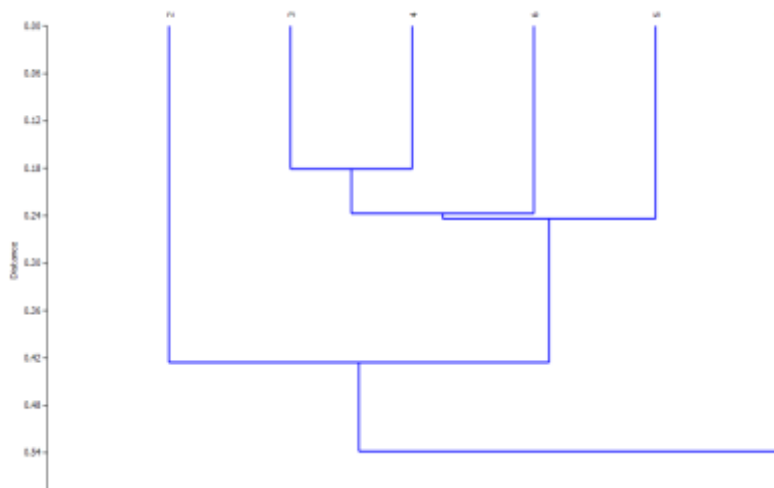


Fig.1 dendrogram showing the relation of the studied species according to morphological characters

Key of the species studied based on macro morphological leaf attributes

- 1A. leaves arrangement alternate
 1B. Fish decay smell *S.volkensii*
 2B. No fishy smell
 1C.Lanceolate leaves *S.kali*
 2C. Ovate leaves *S.inermis*
- 2A. leaves arrangement Opposite decussate
 1D. Hairy leaves
 1E. Anisocytic stomatal type *S.tetragona*
 2E. Paracytic stomatal type *S.tetrandra*
 2D.Glabrous leaves..... *S.longifolia*

IV DISCUSSION

Radford (1986) stated that 'evidence from plant morphology provided the basic language for plant characterization, identification, classification and relationships'. Thus, vegetative morphological characters especially that of leaf, have been employed as a basis for classification since the early days of taxonomy. In fact, vegetative characters can be unreliable because of the similarity happened between unrelated species, but even so Davis and Heywood (1973) deplored the neglect of morphological characters as one of the most serious errors which delay the achievement of a natural system.

Vegetative morphological characters consider the first step in identification and classification in all the botanical works. Davis and Heywood (1973) indicated that whatever other evidence be employed in the construction of a classification, practical considerations demand that the characters used are expressed morphologically. In spite of the fact of the similarity between unrelated genera in the morphological characters, we cannot neglect these characters in constructing the keys in the different floras and as first step in identification. According to the importance of the vegetative characters, morphological aspects of the studied species in genus *Salsola* are considered and summarized in tables 1,2 ,3 and 4.

From our results, the stem outline can be used to identify *S.kali*, which has tetragonal outline, and *S. longifolia*, which have elliptical outline, while the other four species have circular outline. Leaf characters are more valuable in the species identification as *S.kali* and *S. longifolia*, have lanceolate with piliferous apex in *S.kali* and tubular with pointed apex in *S. longifolia*. The other four species have small orbicular leaves. The micro-morphological characters can distinguish *S.tetrandra* by the paracytic stomata and the rest three species have anisocytic stomata. Hair density and type of hairs have been considered good taxonomical characters.

Stace (1984) stated that leaf microcharacters are more conservative than the gross or macrocharacters, and hence more trustworthy as taxonomic indicators. Thus, the epidermal cells have been examined by both light and SEM to investigate. The epidermal cell characters are categorized into four categories according to Barthlott (1981). The arrangement and shape of cells (1st. Sculpture), the fine relief of the outer cell wall (2nd. Sculpture), thickness and pitting of the anticlinal and periclinal walls (3rd. Sculpture)and finally the phytoglyph which cover the full range of features to be seen on the leaf surface, this term was emphasized by Carr *et al.*(1971). Our results revealed that both *S.kali* has heterogenous epidermal cells which are pentagonal or tetragonal elongated scalariform. The rest of the species have homogeneous, hexagonal isodiametric epidermal cells which are areolate in both *S.tetragona* and *S.tetrandra* and ruminant in *S.volkensii* and *S.inermis*.

The epidermal cell walls vary in thickness in different plants and even in different parts of the same plants. Stace (1984) stated that epidermis with exceedingly thick walls is found in leaves of xerophytic plants. In all the studied *Salsola* the epidermal cells have thick walls, except *S.tetrandra* and *S.inermis* with channeled boundaries and convex periclinal wall, except *S.kali* which have raised cell boundaries with flat periclinal wall.

The density and shape of wax deposition varied within the studied species, it was either granulate or flake with different densities.

The shape, size, distribution and orientation of the stomata are all characters which are of taxonomic value. In our study the stomatal index and type have been noticed and found that *S.tetrandra* has the least stomatal index while *S.inermis* has the highest value. The stomata are either anisocytic or paracytic with kidney shaped, small guard cells.

All the studied species enriched with multicellular pointed or tabular hairs with normal or rosette basal cells, except *S.longifolia* which is glabrous, with different densities and lengths. The hairs have echinate walls, except *S.kali* has unicellular hairs with smooth walls and without basal cells. The ANOVA test expressed the results obtained as both the stem width and leaf length is highly significant between the studied taxa while the stem length is significantly different and leaf width is insignificant. The resulted dendrogram separate *S.kali* as expected from all the data and in accordance with all the taxonomic revisions of the genus. *S.longifolia* has its distinct morphological characters as well while the other four species which clustered together shared most of the characters. This group of the four species separate *S.inermis* which has different leaf shapes from the others and *S.volkensii* which has characteristic leaf micromorphological characters and kept *S.tetragona* and *S.tetrandra* very closely related.

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