# Pollen morphology and Stem anatomy of some Salsola L. species (Chenopodiaceae) in southern Iraq

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الصفات المظهرية لحبوب اللقاح و الصفات التشريحية للساق في بعض أنواع الجنس (Chenopodiaceae) Salsola L.

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# ABSTRACT

The pollen morphology and stem anatomy of four Salsola species (Chenopodiaceae) includes S. crassa Bieb., S. jordanicola Eig., S. longifolia Forsk. and S. vermiculata L. from the southern Iraq were studied.

The results of the present study demonstrated that the pollen grains of the four species were characterized by isopolate, spheroidal, polyporate, circular pore and nano-spinulate ornamentation. The pollen diameter, pore number, pore diameter, average of the distance between the center of the pores and C/D ratio that can play an important role in species separation. Pollen diameter and pores number have been shown to give a good taxonomic significance to divide the species in tow groups. *S. crassa* have been largest pollen grains and pore numbers than with other three species that had been studied.

The results of the stem anatomical features investigation provide really significant taxonomical value to distinguish the *Salsola* species, thickness of the epidermis, Cuticles, Chlorenchyma Collenchyma, Sclerenchyma and also Xylem row length were important to separate the four species. Pith diameter to stem diameter percentage (P/S) were varied among four *Salsola* species and showed a taxonomical value among the species.

### الخلاصة:

تم دراسة الصفات المظهرية لحبوب اللقاح والصفات التشريحية للساق في أربعة أنواع من جنس . Salsola L. (Chenopodiaceae) هي S. jordanicola Eig. ، S. crassa Bieb. هي Salsola L. (Chenopodiaceae) و . S. longifolia Forsk. في جنوب العراق .

اتصفت حبوب اللقاح في الأنواع الواردة في الدراسة الحالية بأنها كروية الشكل، عديدة الثقوب، الثقوب دائرية و الزخرفة السطحية شوكيه دقيقة، و تبين إن قطر الحبة و عدد الثقوب و قطر الثقوب و معدل المسافة بين مراكز الثقوب كان لها دورا مهما لعزل الأنواع. وأظهرت الدراسة إن قطر الحبة و عدد الثقوب أعطت قيمة تصنيفية لتقسيم الأنواع قيد الدراسة على مجموعتين. و تميز S. crassa عن الأنواع تحت الدر اسه بامتلاكه اكبر حبوب اللقاح حجما و عددا للثقوب.

وأظهرت نتائج الدراسة الحالية إن للصفات التشريحية للساق لها قيمه تصنيفية جيدة، وأعطى سمك البشرة و الأدمة ، و النسيج الكولنكيمي ، النسيج الكلورنكيمي و النسيج السكلرنكيمي وطول صفوف الخشب أهمية جيده لعزل الأنواع الأربعة . و تبين إن نسبة قطر اللب/ قطر الساق تغاير وأعطى قيمه تصنيفية مهمة لعزل الأنواع .

#### INTRODUCTION

The genus *Salsola* L. is one of the largest genus of Chenopodiaceae comprising more than 130 species, which are generally distributed in the saline marshes (Tutin *et al.*,1964; Heywood, 1978), in the Middle East, central of Asia, Africa, Europe, Australia and North America (Wilson, 1984; Pyankov *et al.*, 2001 and Ke *et al.*, 2003).

About 23 species recorded in Iraq distributed in most regions of Iraq (Al-Rawi, 1964), the name refers to the salty taste and habitat (Glledhill, 2008). In other hand, *Salsola* species, also several species of Chenopodiaceae family have been causing allergy diseases (Ianovici, 2008), such as *Salsola kali* pollen is an important cause of allergic respiratory symptoms in the West and Central states of the USA, in North Africa (Colas *et al.*, 2005) ,and also in Arabian Gulf region (Al-Dowaisan *et al.*, 2004).

Pollen morphological and anatomical features play an important role in plant classification, where to contribute in illustration complexity on relationships between species, it is also reinforce the dependability of the other characters in plant separation; therefore, many studies tend to use pollen morphological and anatomical characteristics and other features of Chenopodiaceae species, because the classic taxonomy in this family is very difficult (Balaei *et al.*, 2004) and numerous species of this family are closely related and form a monophytic group (Shepherd and Wilson, 2007).

Pollen identification and classification are important not only for palynologists, but also for systematists (Zhou *et al.*, 2007). The pollen morphology is not affected by environmental conditions; hence it has been used as a reliable diagnostic key in the taxonomical researches (Gunes and Aytug, 2010), also palynological studies have been carried out on many plants to understand the pollen morphological features of different taxa and the intrapopulational variations of some species (Perveen, 1999); hence, many studies tend to depend pollen characters such as pore shape, pore numbers, aperture location in pollen and ornamentations of exine ((Bhattacharyya, 2005; Pinar, 1999 and Gunes and Aytug, 2010). Several studies were investigated the pollen morphology in some genera of Chenopodiaceae in Iraq (Shreef, 1995; Lefta, 1996).

In addition, Pinar and Oybak (1997) and Toderich *et al.* (2010) were studied the pollen morphology of the *Salsola* species, and other Chenopodiaceae genera were scanned such as *Atriplex* L. Species (Pinar and Inceoglu, 1998a), *Seidlitzia* Bunge, *Aellenia* Ulbrich., *Noaea* Moq., *Cyathobasis* Aellen., *Petrosimonia* Bunge (Pinar ,1999).

Anatomical characters provide better attestation in plant taxonomy on both families and species, several investigations carried out on the plant anatomy showed that the anatomical characters provide significant systematic value (Heywood, 1968; AlMusawi, 1979; Stace, 1981; Al- Mayah, 1983; Haddad, 1987; El- Edany, 1996), also other studies dealing with the anatomical characters in some *Salsola* L. species including (Botschantzev, 1974; Olesen, 1974; Pyankov *et al.*, 1997; Pyankov *et al.*, 1999; Vozneseskaya *et al.*, 1999; Vozneseskaya *et al.*, 2001a; Vozneseskaya *et al.*, 2001b).

As a result of the wide distribution and a large member of *Salsola* species in Iraq, this article aims to investigate the pollen morphology and anatomical features in stems of four species of *Salsola* (*S. crassa*, *S. jordanicola*, *S. longifolia* and *S. vermiculata*) in southern Iraq.

#### MATERIALS AND METHODS

The present study has been carried out during the period February, 2009 to November, 2010 on four species of *Salsola* (Chenopodiaceae) which dispersed in southern Iraq; this study accredited fresh specimens which collected from fields.

Pollen grains were prepared for light microscopy with the methods described by Perveen and Qaiser (2007).the pollen grains were mounted in unstained glycerin jelly. Pollen diameter, aperture diameter, pore numbers and exine thickness were measured; on 20-25 readings from each specimen. The terminology used is in agreement with Erdtman (1971).

The anatomical study of stems is based on the samples collected from the field. Stems from about 25 fresh specimens of 4 species were examined, and then pieces of (1 cm) length of the stems were taken and fixed in FAA. After 24 hours stem pieces were removed and washed with distilled water (Mehrabian et al., 2007), after that put in series of Ethanol (70%; 80%; 90%) respectively about 2 hours for each concentration, then put in absolute Ethanol for 24 hours. Then pass through series of absolute Ethanol and xylen (3:1; 1:1 and 1:3) respectively for 1 hours of each concentration, after that put in absolute xylen for 24 hours. Then the specimens were putted in the mixture of paraffin wax and xylen in 60° for 6 hours, then placed in container contain pure paraffin wax in oven on 60° for 48 hours, then transfused to the block they have been containing paraffin wax with 60° then numbered the sample and abandon to cooled for 24 hours, then were sectioning with thick 20 µm by rotary microtome. The sections were stained with Safranin and Fast Green and then mounted on slides by using Canada balsam. After that the sample were examined and photographed with the camera which situation on Olympus microscope. The anatomical results were according to the (Esau, 1965; Radford et al., 1974 and Fahn, 1982).

Species	Specimens No.	Date	location specimens	
S. crassa	419	8/ 4 /2010	Shatrah	
	425	21/10/2010	Nassiriyah	
S. jordanicola	416	7/2/2009	Nassiriyah	
	410 7/2/2009 Nas		Nassiriyah	
	427	3/2/2010	Bateha	
S. longifolia	421	21/10/2010	Nassiriyah	
	408	7/2/2009	Bateha	
S. vermiculata	427	3/ 2/ 2010	Bateha	
	415	8/4/ 2009	Shatrah	

Table 1: Specimen Characters of four species.

# **RESULTS AND DISCUSSION:**

The results of the present study of pollen grains summarized in (Table 1; Figure1), and showed that the pollen grains in the species *S. crassa* characterized with isopolate; spheroidal; polyporate; nano-spinulate ornamentation , pore circular, Pollen diameter: (28.3- 34.5)  $\mu$ m; pore number: (38- 46); pore diameter: 2.8  $\mu$ m; average of the distance between the center of the pores 8.9  $\mu$ m; C/ D ratio 0.2904,.

The S. Jordanicola pollen grains, commonly isopolar; spheroidal; polyporate; nano-spinulate ornamentation. Pollen diameter: (21.8- 24)  $\mu$ m; pore circular; pore number: (24- 30); pore diameter: 3.8  $\mu$ m; average of the distance between the center of the pores 7.9  $\mu$ m; C/ D ratio 0.3495.

The pollen grains of *S. longifolia* species, in generally, were isopolar; spheroidal; polyporate; nano-spinulate ornamentation. Pollen diameter:  $(17.2-20) \mu m$ ; pore circular; pore number: (30-36); pore diameter:  $3.6 \mu m$ ; average of the distance between the center of the pores 6.2  $\mu m$ ; C/ D ratio 0.3179.

The pollen grains in S. *vermiculata* species, are isopolar; spheroidal; pentopolyporate; nano-spinulate ornamentation. Pollen diameter: (19.3-21.2)  $\mu$ m; pore circular; pore number: (31- 38); pore diameter: 3.1  $\mu$ m; average of the distance between the center of the pores 6.4  $\mu$ m; C/ D ratio 0.309.

the pollen morphology were a good efficient in species separation, and can play an important role in the formation of natural groups and help in the assessment of taxonomical relationship between (Meo and Khan, 2006), because of the pollen morphology is not affected by environmental conditions; therefore, it has been used as a reliable diagnostic features in the taxonomical researches (Gunes and Aytug, 2010), results of the present study gives essentially significant differences among the *Salsola* species (*S. jordanicola, S. longifolia*, *S. vermiculata* and *S. crassa*), in addition it can he used in identification the studied species. Generally, pollen morphology features of all species that had been studied were spheroidal; radial symmetrical; isopolar; polyporat. These findings are also agree with those of Pinar and Oybak (1997) who have reported same features in *Salsola* species , also some studies showed that the similarity in pollen morphological features with the other Chenopodiaceae genera as *Halanthium* Aellen.; *Noaea* Moq. ; *Atriplex* L.; Suaeda L. (Haddad, 1997; Pinar and Inceoglu, 1998a; Pinar and Inceoglu, 1998b; and Pinar, 1999).

Results of this study showed the pollen diameters; pollen numbers and C/D ratio give important value in *Salsola* species separation, which have been shown a broad variation in pollen diameters between *Salsola* species that examined, *S. crassia* is separate with the largest pollen grains (28.3- 34.5)  $\mu$ m, than with *S. longifolia* between (17.2- 20)  $\mu$ m. this feature correlated with the numbers of factors, especially polyploidy (Zera and Keshararzi, 2007),which have been found in members of Chenopodiaceae (Gomurgen and Altinozlu, 2005), such as in *Salsola* species (Goldblatt and Johnson ,1991). This agrees with some researches that referred the pollen size can be used in the *Sasola* species separation (Pinar and Oybak, 1997; Toderich *et al.*, 2010) and other Chenopodiaceae members, for example *Atriplex* and *Sueada* (Pinar and Inceoglu, 1998a; Pinar and Inceoglu, 1998b).

The pore numbers are usually used as one of diagnostic features in pollen analytical and taxonomic study of Chenopodiceae taxa (Pinar and Inceoglu, 1998b), the results explain that the pore numbers also showed significantly varied between *Salsola* species that studied, *S. crassia* characterized with the largest pore numbers were (41) pore compare with lowest pore numbers were recorded in *S. Jordanicola* (29) pore, there is no relationship between the pores number and pollen diameter, but the results show association between pores diameters and pores number, when the pore diameter was small the pore number increased, these results agree with Pinar and Oybak (1997) who give the same inference in pollen grains of *Salsola* in Turkey.

The C/D ratio is one of the most diagnostic features of the family Chenopodiaceae used in pollen analytical and taxonomic investigations (Pinar, 1999), it had shown important taxonomical value and appeared variation among all species were examined. *S. jordanicola* has been the largest C/D ratio was (0.3495), while the lowest ratio (0.2904) was in *S. crassa*, these results agree with (Toderich *et al.*, 2010) who referred to the C/D ratio had given significant values in *Salsola* spices. The wall thickness in pollen grains also show variations among four species, *S. crassa* appeared with thicker walls of pollen grains than the other species. The results also showed significant variation in the pollen diameters and that have been given an important role in dividing the species into two kinds on the pollen diameter foundation:

1- Small size pollen grains, the diameter between (17.2-24) µm, include:

S. jordanicola; S. longifolia and S. vermiculata.

2- Medium pollen grains, the diameter between (28.3- 34.5) µm in S. crassa.

Present anatomical investigation provides really significant taxonomical value among the four *Salsola* species; results of the anatomical studied were summarized in (Table 2 and Figure 2, 3). Generally, the epidermis in all studied species was a uniseriate with square; triangular and elliptic shapes and the epidermal layer thickness show variation among most species that were studied, S. *crassa* was characterized with largest thick epidermal layer (12.2-16.5) µm while *S. longifolia* has lowest epidermal layers thickness was (8.7-10.8) µm. Cuticle layers demonstrate diversity in *Salsola* species which scanned too, unto in the same species ,generally *S. crassa* has thicker cuticle layers than with the other species, the thick of this layer has been evidenced for the xerophytes (Fahn, 1982), and effected by many factors such as genetic factors (Al-Mayah,1983), and environmental factors (Krings, and Kerp, 1998).

Cortex was divided into two regions, the first 1-2 layered thin walled, elongated were Chlorenchyma cells located outside the cortex under the epidermis, which capable of photosynthesis, this layer found in stem of many plants (Rudall, 2007), it is similar to these of findings of Keshavarzi and Zare (2006) in some Chenopodiaceae species such as *Arthrocnemum macrostachyum* (Moric) C. Koch. and *Halocnemum strobilaceum* (Pall.) M. Bieb. Collenchymas tissues were alternated with the Chlorenchyma tissues included between 4-5 layers of spherical and rectangular Collenchymas cells, which covering with an angular walls thickness and located under the epidermal layers. After that, Parenchyma tissues has been contained of 5- 6 layers of a large, thin wall parenchyma cells, Bercu and Bavaru (2004) have showed the similar features on the Cortex of *Sasola kali* L.

Then, the Sclerenchyma tissue contains of 2-4 layers of fibers construct a ring separate between the Cortex and Vascular bundles. This layer of fibers which are given a good taxonomical importance to split the *S. longifolia* with thicker fiber layers was (51.5-68.1) µm than with the *S. crassa*, *S. jordanicola* and *S. vermiculata* were (44.2-59.6), (46 -63.7), (45.8-71.3) respectively.

Druses crystals were dispersed in the Cortex, especially in *S. crassa* including a large number of crystals, while *S. vermiculata* and *S. Jordanicola* lack crystals, Cortex ended by endodermis were consist of one layer of round, square and rectangular cells with thick walls, followed by Pericycle, which consist of one layer thin walled cells.

The collateral vascular bundles which form a ring around the Pith under the Cortex, Phloem shows a variable in member thickness of four species, *S. longifolia* had the largest members mean by (53.2)  $\mu$ m compare with the other species that had been studied, especially in *S. vermiculata* contains small members by average (36.3)  $\mu$ m., the vascular cambium that located between phloem and xylem tissues, that had been contained of (3- 4) series of small, thin wall and conglutination cells. These results agree with Ramazannejad *et al.* (2006) who deal with the stem anatomy of some species belong to the genera *Anabasis* L., *Haloxylon* Bge., *Hammada* Iljin, *Seidlitzia* Bge. ex Boiss. of the tribe *Salsoleae* (Chenopodiaceae).

Xylem arranged in parallel rows, which show varied in rows length between the present species, *S. crassa* characterized with longer xylem rows were (116)  $\mu$ m compared with the other species, while in *S. vermiculata* were a short rows (83)  $\mu$ m. In addition, the diameters of treachery members showed that the difference between the species examined (Table. 2), usually the *S. vermiculata* contains a large treachery member, whereas in *S. jordanicola* the treachery members were small. The Vascular bundles enclosed by 1- 2 layers thick walled Sclerenchyma cells.

The Pith located in the stem center contained from a big size, spherical and elliptical shapes parenchyma cells (Fig. 2), the diameters of pith regions had some difference among studies species and sometimes in the same species, generally, the pith regions were a large in four *Salsola* species, these indicated to the desert habitat of plants (Anderson and Creech, 1975), with that the *S. longifolia* has been a largest percentage of pith regions was 48%, while *S. crassa* has a lowest percentage of pith regions was 31%. In addition, results show the starch grains find out in parenchyma cells of pith region.

The present study shows that the Pollen diameter, pore number, C/ D ratio and stem anatomy especially Chlorenchyma layers, fibers and pith have been given a good value to species separation and understandable the relationship between species.

this family has the greatest diversity in anatomy among dicot families (Vozneseskaya *et al.*, 2008), different factors may influence diversity among Chenopodiaceae species (Shepherd *et al.*, 2005), includes species complexes ,hybrids, also polyploidy (Shepherd and Yan ,2003), and also exhibits diversity in habitats, life forms, and photosynthetic characters in assimilation organs is particularly complex in *Salsola,* and related genera in tribe Salsoleae (Pyankov *et al.*, 2001)



Figure 1: Pollen grains of the *Salsola* species stems: (A) *S. crassa*, (B) *S. Jordanicola*, (C) *S. longifolia* and (D) *S. vermiculata*, (10µm).



Figure 2: Cross section of the Salsola species stems: (A) S. crassa,
(B) S. Jordanicola, (C) S. longifolia and (D) S. vermiculata. (100 μm) EP: Epidermis - CO: Cortex– PH: Phloem- XY: Xylem - PI: Pith.



Figure 3: Salsola species stems: (A) S. crassa, (B) S. Jordanicola,
(C) S. longifolia and (D) S. vermiculata. (100 μm)

Species	Pollen Diameters	Pore	Pollen Wall		Pore	C*	C/D ratio	
Opecies	(D) Numbers		Exine	Entine	Diameters	Ŭ		
S. crassa	(28.3- 34.5) 30.7	(38- 46) 41	1.4	0.7	2.8	8.92	0.2904	
S. jordanicola	( 21.8- 24) 22.6	(24-30)29	1.3	0.4	3.8	7.9	0.3495	
S. longifolia	( 17.2- 20) 19.5	( 30- 36) 34	1.3	0.6	3.6	6.2	0.3179	
S. vermiculata	(19.3-21.2) 20.7	( 31- 38) 36	1.2	0.4	3.1	6.4	0.309	

Table 1: Pollen grains diameters of some Salsola species ( $\mu m$ ).

C\* Mean of the distance among the center of three pores.

Table 2: Anatomical characters of the stems oF some Salsola species.

Species	Epidermis Thickness (µm)	Cuticles thickness (µm)	Cortex (µm)			Phloem	Vessels Diameter				
			Collenchyma thickness	Parenchyma thickness	Sclerenchyma thickness	Xylem row length (µm)	Diameter (µm)	means (µm)	*P (mm)	**S (mm)	P/ S %
S. crassa	( 12.2-16.5) 14.2	3.1±0.2	( 32.5-40.2) 37	( 19.8-28.2) 25	( 44.2-79.6) 57	(98.3-126.4) 116	( 23.4- 38) 31.9	27. 3± 3.7	0.62±0.2	2± 0.1	31
S. jordanicola	( 9.4-14.2) 13.1	1.9± 0.4	( 26.3-32.7) 31	( 18.1-29.5) 26	( 46 -63.7) 51	(108 -121.7) 115.3	(27.2-35.1) 30.7	35. 1± 5.2	0.97±0.6	2.3±0.5	42
S. longifolia	( 8.7-10.8) 9.2	2.6± 0.2	( 27.1-35) 33	( 35 -40.1) 38	( 51.5-78.1) 64	( 89.6- 121) 109	( 31 -45.5) 37.2	38. 5± 5.9	1.01±0.3	2.1±0.2	48
S. vermiculata	( 10.1-11.9) 11	2± 0.5	( 31.4-37.9) 35	( 27.5-33.9) 31	( 45.8-71.3) 49	( 74.9- 90.1) 83	(25.2–30.3) 28.3	21. 7± 6.4	0.57±0.2	1.7±0.4	33

\* Pith Diameter means (P).

\*\* Stem Diameter means(S).

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