

Implications of Palynological and cytological Data for Distinguishing *Salix* L. Species in Kurdistan Region of Iraq

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Abstract

Palynological and chromosomal characters were systematically used to compare between species of *Salix* growing in Kurdistan Region of Iraq. The investigation revealed that pollen colpi length, exine thickness and surface sculpturing contribute none significantly in species delimitation, while size and shape classes of Erdtman (1945, 1971) respectively, in addition to the occurrence of Parasyncolpate pollen grains are highly valuable characters for distinguishing of the species.

The chromosome counting of the pollen grain mother cells was $2n=38$ for *S. acmophylla* and *S. purpurea*, while $2n=76$ for tetraploids of *S. alba*, *S. babylonica* and *S. aegyptiaca*. According to the results of chromosome counting, *S. acmophylla*, which is closely related to *S. alba* is easily recognized by their differences in chromosome numbers.

Introduction

Pollen morphology provides significant evidences to support the separation of the taxa at different levels of hierarchy. For example pollen provides the primary basis for recognition of subfamilies in the Labiatae, but at the tribal level only the Ajugeae has distinctive pollen. Several genera, notably *Collinsonia*, *Salvia*, *Teucrium*, and *Trichostema*, have pollen that is very different from other genera. At the infrageneric level, pollen provides valuable taxonomic characters in several genera, notably *Hyptis*, *Monardella*, *Salvia*, *Stachys*, *Teucrium*, and *Trichostema* (Abu-Asab and Cantino 1989, 1993).

Vafadar, et al. (2009) studied the pollen morphology of *Amygdalis* L. in Iran, the results showed that Palynology has important useful value for the genus *Pyrus* in studying origin, evolution and taxonomy, the researcher concluded that not only polar axis, equatorial axis and polar axis/equatorial axis ratio (P/E ratio) of pollen grains but also ridge width, distance between ridge edges, pore (pit) diameter and pore (pit) frequency of surface sculpture of pollen will be useful index for identifying species and varieties. Palynologically the genus *Salix* is little investigated and the available information is inadequate to explain species relationships. David and Hogsette (2008) collected and described pollen grains adhering to the exoskeletons of flies acting as pests of human and livestock, all the specimens were identified as Carolina willow, *Salix caroliniana* Michaux. The haploid number of chromosomes in angiosperms, as referred by Jones and Luchsinger (1986), range from $n=2$ in

Haplopappus gracilis to around $n=132$ in *Poa littorea*, but most angiosperms have chromosome number ranging between $n=7$ and $n=12$, on the other hand, the same author estimated number of polyploids as 35% to 40% of flowering plants. Raven (1975b), in an intensive review article, suggests that the basic chromosome number for most angiosperms is probably 7. speculates that an initial burst of polyploidy occurred during the early evolution of flowering plants, since many of the families with primitive features have high chromosome numbers (cited after Jones and Luchsinger, 1986).

Researchers often use chromosome counting for taxa delimitation, at different levels of hierarchy, for instance. Cardoso, et al. (2000) determined Chromosome number in 52 individuals of 14 taxa of *Leucaena* Benth, from 22 populations. Intraspecific variability was found for *L. lempirana*, *L. macrophylla* and *L. shanonii*, and one diploid population of the tetraploid species *L. pallida* was identified. The variability detected in chromosome numbers shows the complexity of the diploid and tetraploid species evolution, and suggests multiple origins for some of the polyploid taxa.

In an early investigation for chromosome numbers, Suda and Argus (1968) reported for 19 species of North American *Salix*, one natural hybrid, and one introduced species. Seventeen species were examined cytologically for the first time, of the 19 native North American species, 11 are diploid, four are tetraploid, one is triploid, one hexaploid, one dodecaploid, and one exhibits more than one ploidal level. According to Suda and Argus (1968), approximately 40%

of *Salix* species are polyploids, ranging from tetraploids to octaploids.

Chromosome count is known for many Asian and European *Salix* (Darlington and Wylie, 1956; Love and Love, 1961; Suda, 1963; Argus, 2009). Barcaccia, *et al.* (2003) insisted that the tetraploidy in *Salix* makes genetic analysis far from easy. For them, nothing is known about willow genomic constitution and whether species are autopolyploid or allopolyploid. Moreover, they added that the cytological observations of the pairing behavior of tetraploid willow, to reveal bivalent or multivalent formation, are difficult owing to their high chromosome number and small chromosome size. Since willows have a high basic chromosome number ($n=19$), species with $2n=38$ may be ancient polyploid derivatives (Stebbins, 1950; Lewis, 1980) that behave as functionally diploids (Aravanopoulos, *et al.* 1993; Triest; *et al.* 1998). No serious investigation of the *Salix* genus was conducted so far, only the contribution made by Mahmud (1983) on the palynology and cytology of *S. alba* and *S. acmophylla* growing in Iraq was valued.

The current investigation is considered as the first contribution to deal with the description of the pollen morphology and chromosome counting of Kurdistan representatives of the total number of *Salix* species of Kurdistan region. It is intended to serve as a reference study along with other studies to clarify variations within and between species.

Material and Methods

Pollen grains:

Polliniferous buds in the stage just before anthesis were obtained from living specimens of *S. alba*, *S. acmophylla*, *S. aegyptiaca*, *S. purpurea* in their natural habitats, and *S. babylonica* in cultivation, during years 2009 and 2010. Five trees from each species were selected such as to cover a wider range of species distribution. Specimens were kept in the dept. of Biology/ University of Zakho, for future reference.

Flower catkins from each tree were isolated and dried in the laboratory conditions. Selected Pollen grains were then kept at 4°C for later use.

Pollen grains for LM observations were made using the standard acetolysis method of Erdtman (1952). Samples of pollen grains were mounted in fast green-glycerine jelly. Morphological observations, including polar length (P) and

equatorial diameter (E), P/E ratio number of apertures, mesocolpium (in the equatorial view), apocolpium, colpus length, exine thickness, apertural shape, were made using a Zeiss light microscope. The mean, average, and standard deviation for each character was based on 40 observations.

Size classes were assessed according to Erdtman (1945), while the shape classes were followed according to Erdtman (1952). The pollen terminology in general follows Punt *et al.* (1999) and Erdtman (1971).

Chromosome number:

Fresh male flower buds were collected from each species in their natural populations located at different physiographic regions in order to collect greatest possible variation with the exception of *S. babylonica* which exists only in cultivation around water sources, in parks or landscape. Catkin collection was made during 2010. Specimens with their vouchers were deposited in the dept. of Biology/ University of Zakho.

During buds collecting, the entire catkin was gathered when anthers were already formed before turning yellow. A freshly prepared Carnoy's solution was used. The buds were dropped into the fixing solution in the field, left at room temperature for 20-24 hours. Then the buds were removed from the solution, washed twice with 70% ethyl alcohol to remove any remaining acetic acid, and then placed in 70% ethyl alcohol before then keeping in a refrigerator at 4°C . The promising buds were selected by needle, placed on a glass slide, in 1 or 2 drops of acetocarmine to stain the chromosomes. The buds were squashed with the smooth end of a glass rod, covered with a slide and tapped gently. The slides were heated gently for a short time avoiding boiling, pressed by using filter paper and thumb. The mounted material was observed using light microscope (Axiostar, Japan). The chromosomes were studied under oil immersion at magnification of 1000x.

Results and Discussion

Pollen characters for the genus:

The results showed that Palynology has important useful value for the genus *Salix* in studying taxonomy (table.1). Pollen grains are oblate to prolate, very rarely suboblate or perprolate, tricolporate, isopolar, radially

symmetrical, size: Polar length P 15.6-27.6 μm , and equatorial diameter E 13.8-19.6 μm , P/E ratio: 0.91-1.60. Colpi length 7.9-19.8 μm , all are long, narrow, reaching to the poles; pores are mostly indistinct, appearing as a thinned area within the copal margins. Apocolpium ranges 13.9-24.1 μm . Mesocolpium ranges 8.9-17.7 μm . Exine ranges 0.9-2.8 μm thick, two-layered, nexine about as thick as sexine. Tectum surface is reticulate, heterobrochate, reticulum is finer at poles and along colpi margins, lumina are variable in shape and size ranging from 1.1 to 4.1 μm .

Pollen characters for the species

Statistics presented in table (1) show little differences in polar axis and equatorial diameter between species; longest polar axis and equatorial diameter are scored in *S. alba* while smallest in *S. purpurea*. The smallest P/E ratio is found in *S. aegyptiaca* while highest in *S. babylonica*.

According to the size classes of Erdtman (1945) based on the largest grain axis, the grains of both *S. purpurea* and *S. aegyptiaca* are considered small or minutae, while those of *S. alba*, *S. acmophylla*, and *S. babylonica* are small to medium (minutae to mediae), with much higher ratio for small-sized grains, as indicated by STD the highest ratio of medium-sized grains is present among those of *S. alba* and *S. acmophylla*.

Shape classes of Erdtman (1971) given in table (2) indicate the occurrence of three classes namely oblate-spheroidal to prolate in *S. alba* and *S. acmophylla*, prolate-spheroidal to prolate in *S. babylonica*, and prolate-spheroidal to prolate in *S. aegyptiaca* and *S. purpurea*.

colpi are longest in *S. alba* while shortest in *S. purpurea*, tapering at both the ends and weakly correlated with the polar length.

Apocolpia in the polar view exhibit minor differences between species with high overlapping of measurements, but the presence of numerous parasyncolpate pollen grains in *S. acmophylla* (figure,3) constitutes a major difference providing significant diagnostic character for separating this species from the rest of *Salix* species. Mesocolpia in the equatorial view, also show slight variability between species with no spatial differences, thus providing non significant taxonomic feature.

Exine is slightly thinner in *S. aegyptiaca* compared with the exine thickness of the other species, sexine is more or less thicker than nexine in all species, but this variability is also insignificant for taxonomic application.

The exine sculpturing is more or less coarsely reticulate and pattern of muri is irregular (figures, 3), reticulation is more distinct at the center of the mesocolpia in the equatorial view than in the polar view. Lumina are variable in shape and size, often circular and or angular; lumina size gradually reduced towards the colpi forming the colpus membrane. Sculpturing of *S. alba* is distinctly reticulate, relatively coarser than other species, 1.7-4.1 μm in diameter, finest lumina are found in *S. aegyptiaca* and *S. purpurea*, 1.3-2.7, 1.1-3 respectively.

Pollen ectoaperture and endoaperture structures showed also small degree of variations among the species examined compared to the other diagnostic criteria. Ectoaperture margins are indistinct but sometimes distinct.

By depending on the pollen outline, it is also obvious that polar view for all *Salix* species are the spherical, ovate and triangular shapes, no elliptic shapes is found. Nearly half of grains of *S. acmophylla* are triangular, a character may be useful for taxonomic significance. In equatorial view, the ovate, elliptic, and spherical shapes are common; no triangular is present in any of the species (table,2).

It is obvious from the results that some of the pollen structures and measurements seen by LM can be relied in this kind of delimitation; not only polar axis, equatorial diameter and polar axis/equatorial diameter ratio (P/E ratio) of pollen grains but also the presence of parasyncolpate pollen grains and the shape of the pollen outline in the polar and equatorial views.

Pollen colpi length, exine thickness, pore structure, and the exine surface sculpturing are inadequate in discriminating between species. Indeed, the *Salix* species are found to exhibit high similarity in terms of the overall pollen morphology; pollen surface reticulation, colpi length and structure, number and structure of pores, mesocolpium dimension, exine thickness, and these resemblances shows close relationships and are in support of the monophyletic origin of the genus.

Based on the results displayed above the following taxonomic groups may be recognized:

A. Size

1. Pollen grains Minutae; P usually <24.7 μm : *S. aegyptiaca*, *S. purpurea*

2. Pollen grains Minutae -Mediae; P usually $>24.7\mu\text{m}$: *S. alba*, *S. acmophylla*, *S. babylonica*.
 3. Prolate-spheroidal to prolate in *S. aegyptiaca* and *S. purpurea*.

B. Apocolpium

1. Parasyncolpate pollen grains present in which the Apocolpial field delimited by the margins of the anastomosing colpi: *S. acmophylla*
 2. Parasyncolpate pollen grains not present, the Apocolpial field not delimited by the margins of the anastomosing colpi: *S. alba*, *S. babylonica*, *S. aegyptiaca*, *S. purpurea*.

Table (1): Pollen grain quantitative and qualitative character.

Character		<i>S. alba</i>	<i>S.acmophylla</i>	<i>S.babylonica</i>	<i>S.aegyptiaca</i>	<i>S.purpurea</i>
Polar axis=P (μm)	Mean	21.54	21.03	20.89	20.68	19.92
	STD	± 3.41	± 3.34	± 2.88	± 2.10	± 2.38
	Range	15.8-27.6	16.7-26.8	15.6-25.8	18.1-24.7	16.4-23.9
Equatorial diameter= E (μm)	Mean	18.09	16.88	16.33	17.37	16.00
	STD	± 1.17	± 1.39	± 0.90	± 1.07	± 1.05
	Range	16.1-19.6	14.5-18.9	14.6-17.8	15.2-18.9	13.8-17.5
Colpus length (μm)	Mean	16.02	13.61	13.80	14.26	11.40
	STD	± 2.58	± 2.39	± 3.07	± 2.76	± 2.75
	Range	11.4-19.8	10.8-17.5	8.8-18.7	9.7-17.8	7.9-15.5
Mean ratio= P/E	Mean	1.20	1.23	1.27	1.18	1.24
	STD	± 0.19	± 0.16	± 0.15	± 0.10	± 0.13
	Range	0.91-1.6	0.91-1.56	1.01-1.56	1.03-1.39	1.03-1.48
Apocolpium	Mean	19.22	17.65	18.90	18.44	19.57
	STD	± 2.91	± 1.87	± 1.62	± 2.97	± 3.03
	Range	15.1-23.3	14.5-20.9	16.3-21.5	13.9-22.3	15.5-24.1
Mesocolpium	Mean	14.47	12.56	12.60	15.10	13.48
	STD	± 2.34	± 2.29	± 1.76	± 1.89	± 2.66
	Range	11.2-18.1	9.8-16.4	10.2-15.6	12-17.7	8.6-16.8
Exine thickness	Mean	1.88	1.75	1.76	1.55	1.78
	STD	± 0.53	± 0.41	± 0.21	± 0.22	± 0.31
	Range	1.2-2-8	0.9-2.3	1.4-2	1.2-1.9	1.3-2.2
Lumen diameter	Mean	2.50	2.11	2.22	2.06	1.91
	STD	± 0.65	± 0.60	± 0.57	± 0.43	± 0.57
	Range	1.7-4.1	1.5-3.2	1.2-2.9	1.3-2.7	1.1-3.0
Size classes		Minutae- mediae	Minutae- mediae	Minutae- mediae	Minutae	Minutae
Shape classes		Oblate spheroidal to perprolate	Oblate spheroidal to perprolate	Prolate- spheroidal to perprolate	Prolate- spheroidal to prolate	<i>Prolate- spheroidal to prolate</i>

Table (2): Pollen grain outline shapes in the polar and equatorial views.

Pollen view	Pollen outline shape	<i>S. alba</i>	<i>S. acmophylla</i>	<i>S. babylonica</i>	<i>S. aegyptica</i>	<i>S. purpurea</i>
Polar view	Spherical	***	**	*	***	***
	Ovate	**	*	****	***	***
	Elliptic	-----	-----	-----	-----	-----
	Triangular	**	****	**	**	*
Equatorial view	Spherical	*	**	**	***	*
	Ovate	***	***	**	**	****
	Elliptic	***	***	**	**	**
	Triangular	-----	-----	-----	-----	-----

Note: * = 15-25, ** = >25-35, *** = >35-45, **** = >45.

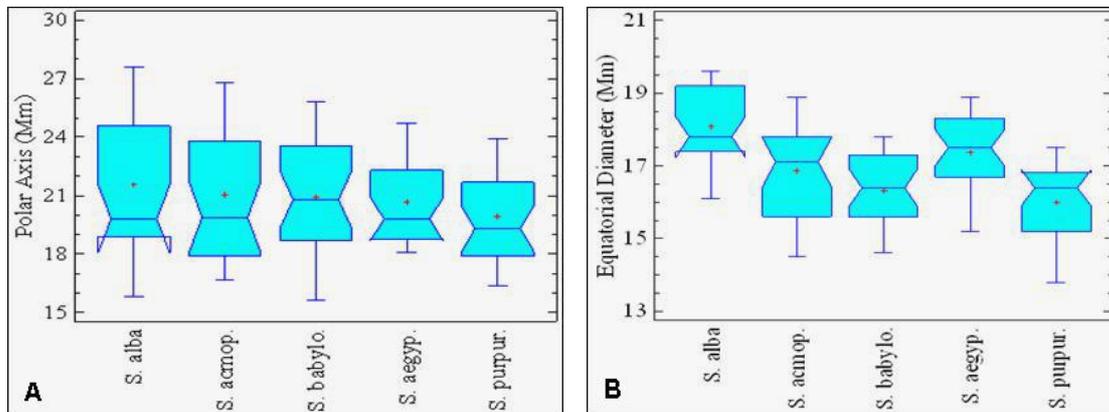


Figure 1. Variation in: A. Polar view, B. Equatorial view.

Note: The shaded area includes 50% of observations, the horizontal line within the shaded area is the median, and the plus sign (+) is the average of the observations.

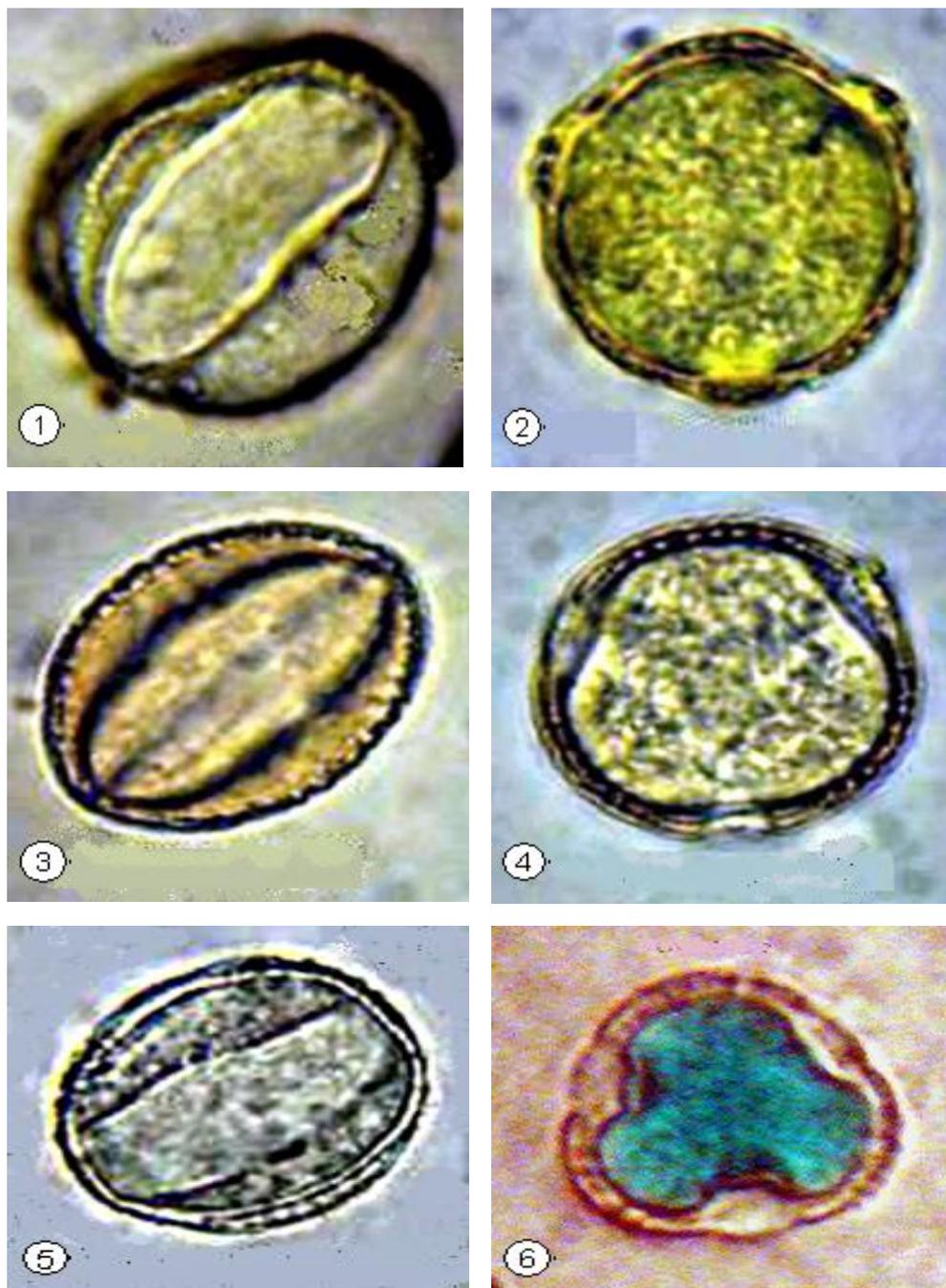


Figure 2. *S. alba*: 1. Equatorial view (2760x), 2. Polar view (2320x), 3. *S. acmophylla*, equatorial view (2960x), 4. Polar view (1900x). *S. babylonica*: 5. Equatorial view (2750x), 6. Polar view (1810x).

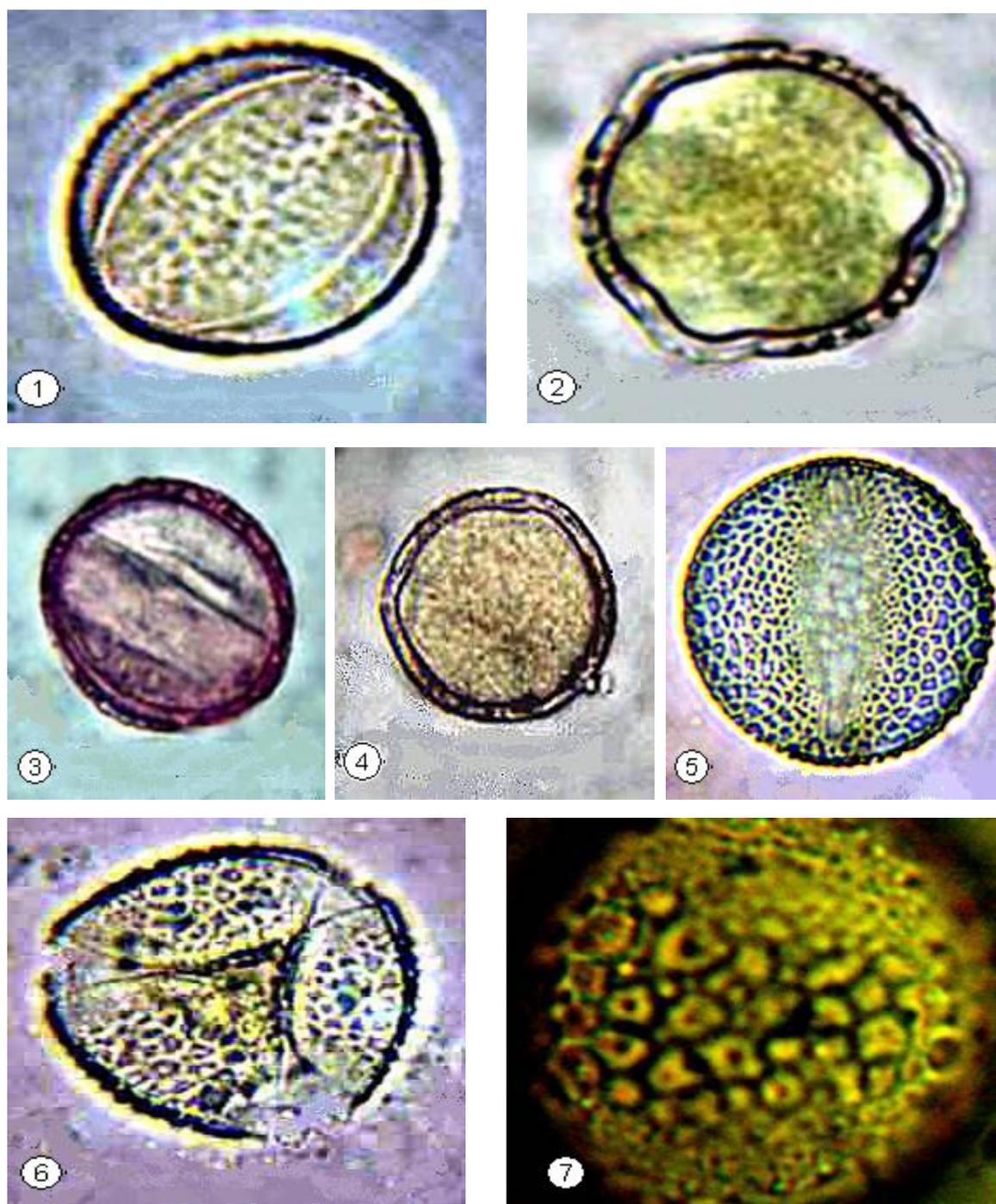


Figure 3. *S. aegyptiaca*: 1. Equatorial view (2590x), 2. Polar view (2410x), 3. *S. purpurea*, equatorial view (2310x), 4. Polar view (1750x), 5. *S. acmophylla*: Equatorial view showing lumina and muri of the exine reticulum which vanish over the colpus (2200x), 6. Polar view of a Parasyncolpate pollen grain (1850x), 7. *S. alba*: reticulum of the exine surface (1400x).

Chromosome number and characteristics:

A study of the meiotic divisions in the developing flowers of the species has proved to be valuable, but it can, of course, only be obtained during a very limited periods, in the early spring. These periods differ from species to species. Although, the representation members of our willows are found to root very easily in an incubator or water at 20 to 30 °C at practically any time of the year but most favorably is from February to the beginning of April. Chromosomes of the somatic complement, from the root tips, didn't yielded encouraging results. This is in contrary to the results obtained by Wilkinson (1934), who observed chromosomes of the somatic complement, from the root tips, of willows growing in UK to be valuable in the recognition of *Salix*.

Salix alba

The white willow is of widest area of distribution and highest degree of variability forming dense population of riparian thicket. In some suitable localities the plant attains very large size of spreading branches with a girth exceeding 2m.

With the exception of *S. acmophylla* and often *S. purpurea* all *Salix* species are polyploids. Concerning *S. alba*, it is a tetraploid with the basic chromosome number of $x=19$. The results of chromosome counting for the pollen grain mother cells is $n=38$, while $2n=76$. The haploid chromosome number $n = 38$ reported here confirms the result of some researchers (Iliev, 1992; Ferakova, 1974; Vachova, 1978; Drušković, 1995). While Wilkinson, 1934; Fedorov, 1969; Barcaccia, *et al.* 2003; and Petrova, *et al.* 2007 also reported the same chromosome number in different locations. In contrast to the results of all above mentioned authors, Mehra (1976) reported a diploid chromosome number $2n = 38$ for this species. As figure (4) suggests, all of the 38 chromosomes in the *Salix alba* are small, and the majority appear to be individually indistinguishable on morphological grounds, it has, however, been found that few chromosomes which are almost twice the average length. Few others are short or almost oval, wider than the average, or extremely small.

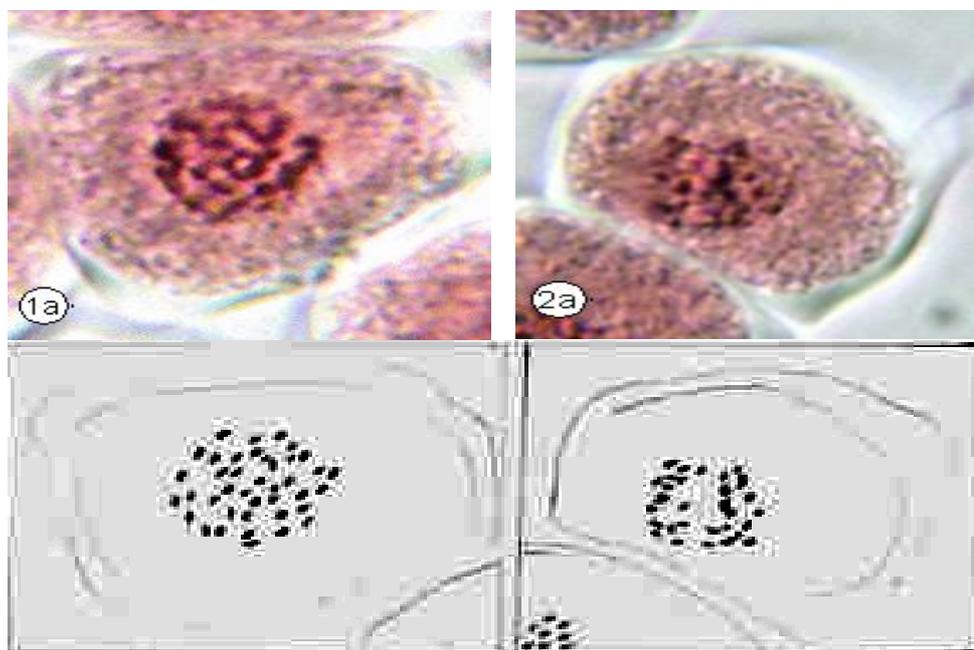


Figure 4. Shows late prophase 1000x of *S. alba*.

Salix acmophylla

The haploid chromosome number of *Salix acmophylla* is found to be $n=19$ (figure,5), while $2n=38$. Very few counts are available for this species in literature, only one count reported by Mahmoud (1983) in Iraq. Our counted

chromosome number is in agreement with that published by the same author. Although, the material investigated was not limited, no cells of more than $2n=38$ were observed. All of the 19 chromosomes in the *S. acmophylla* are moderate in size compared to chromosomes of other

species, and the majority appears to be individually indistinguishable, few of them are larger than the average, some are very small, others are oval in shape. The $2n=38$

chromosome number of *S. acmophylla* together with the relatively large sizes of some chromosomes might be helpful in identifying this species.

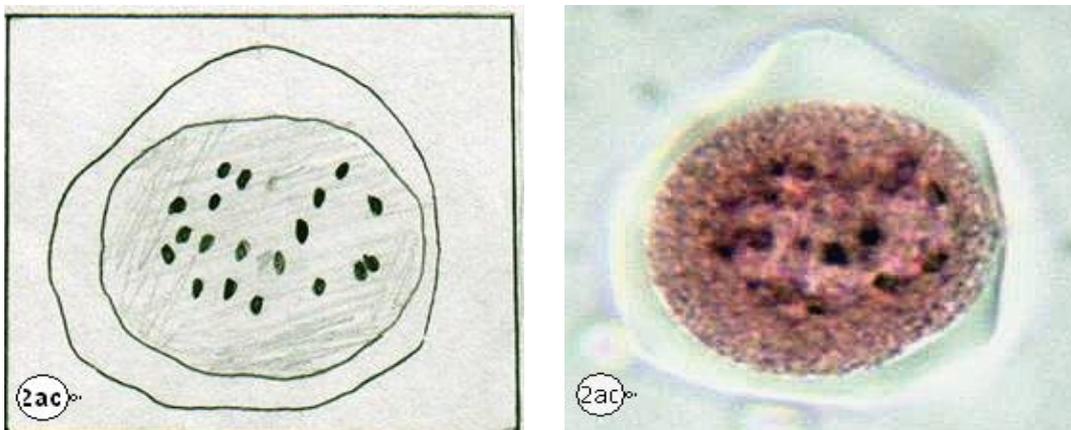


Figure 5. *S. acmophylla*. 2ac (ac=*acmophylla*). Late prophase I, 1600x.

Salix babylonica

The chromosome number found here is $n=38$, it is the haploid chromosome number seen at the meiotic division of the pollen mother cells. The weeping willow is observed to be a tetraploid dioecious tree with $2n=76$. Our count is the first record for this species in Kurdistan and Iraq. This count is in agreement with the results

published by Bowden (1945) and Takayuki Azuma (1999). The great majority of chromosomes are alike in morphology, with few are found to be larger or smaller in sizes. Careful investigation shows greater resemblance between chromosome morphology of *S. babylonica* and *S. acmophylla* in compared with other species as show in figure (6).

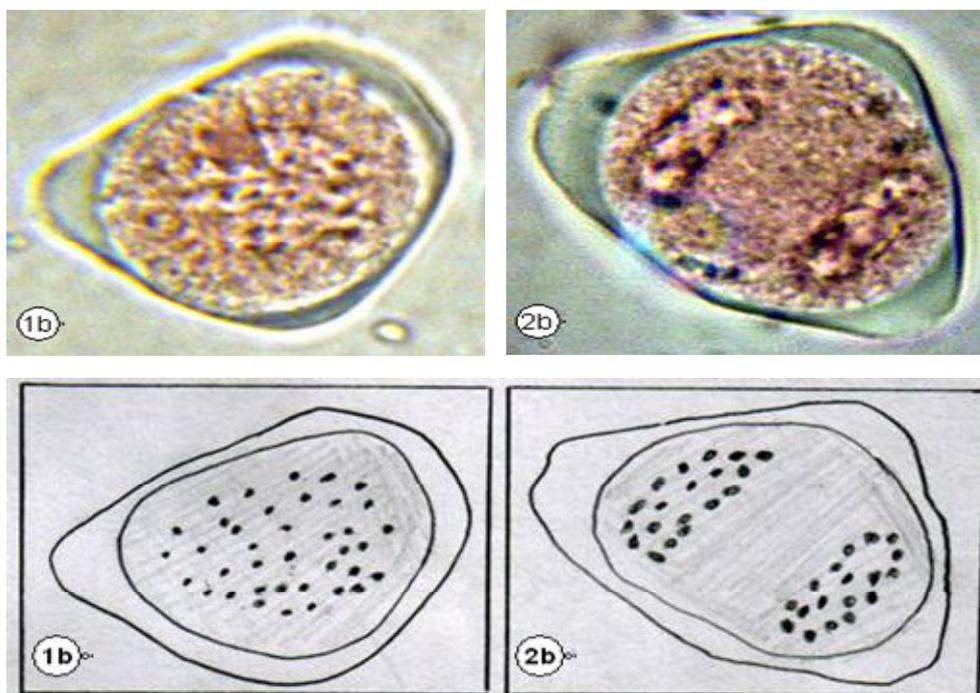


Figure 6. *S. babylonica*. 1b (b=*babylonica*). Metaphase I, 2b. telophase I.

Salix aegyptiaca

The Egypt willow is also a tetraploid dioecious tree (Wilkinson, 1944). The results for the chromosome number of pollen mother cells found to be $n=38$, while $2n=76$.

Like other tetraploid *Salix* species, the basic chromosome number is 19. The count recorded above is considered to be the first in Kurdistan. The haploid chromosome number $n = 38$ reported here confirms the result of Wilkinson (1944 and 1955). Chromosomes of *S. aegyptiaca* are also small, in compare those of *S. alba* and *S. babylonica*, but differ significantly from those of *S. acmophylla*, figure (7).

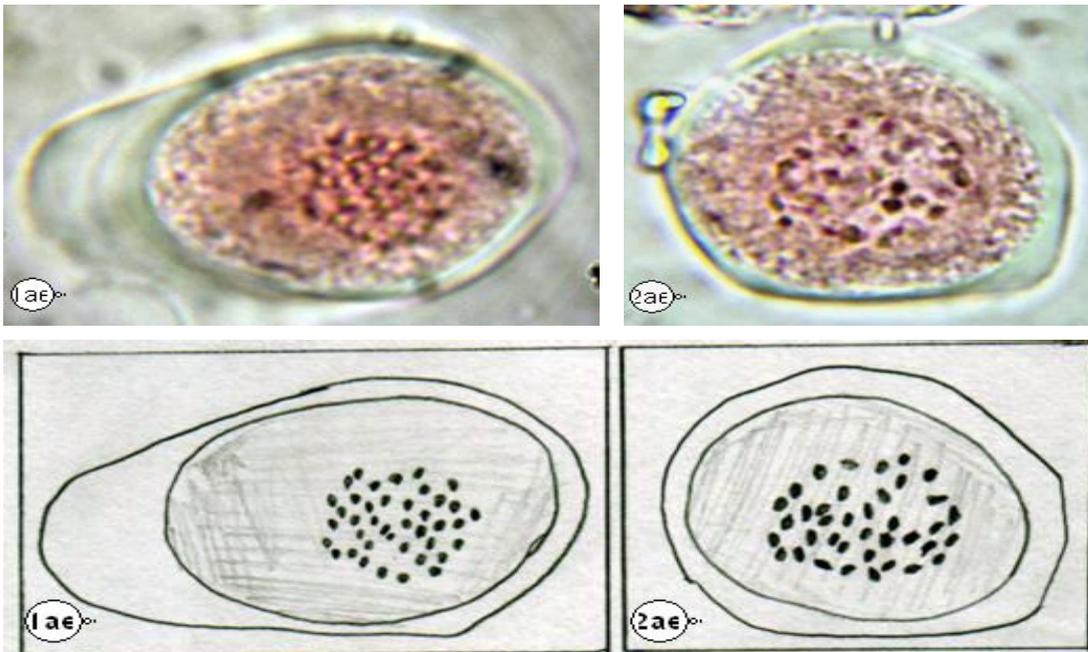


Figure 7. *S. aegyptiaca*. 1ae (ae=*aegyptiaca*). Prophase I, 2ae. Late prophase I. 1000x.

Salix purpurea

The current investigation revealed that the haploid chromosome number for this *ssp* is $n=19$, while $2n=38$. The results of plant morphology and chromosome counting indicate the possibility of separating *S. purpurea* from the rest of species. Figure (8) reveals, that in compared to *S. purpurea* chromosomes are strictly larger and are contrasted with chromosomes of *S. acmophylla*. High variation is encountered between the individual chromosomes.

Results were the first record in kurdistan for this species and confirmed the chromosome count reported by Harrison (1924) and Thibault (1998). All samples tested revealed diploid chromosome number; a result completely differs from that of Ngantcha (2010). According to the later author, the *S. purpurea* lines used in his investigation had higher DNA values than that of Thibault (1998) and thus appeared to be triploids.

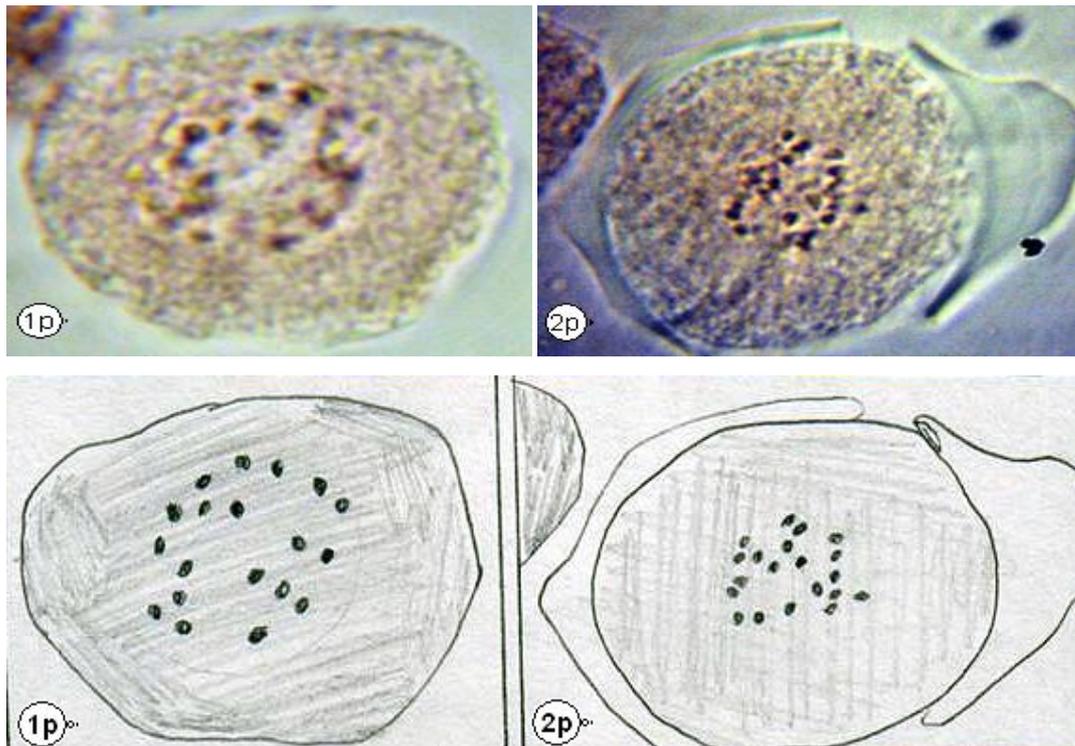


Figure 8. *S. purpurea*. 1p (p=*purpurea*). Late Prophase I, 1600x, 2p. Metaphase I, 1000x.

Table 3. Current and Previous Chromosome Records.

Authors	Species				
	<i>S. Alba</i>	<i>S. acmophylla</i>	<i>S. babylonica</i>	<i>S. aegyptiaca</i>	<i>S. purpurea</i>
Current counting	n=38 2n=76	n=19 2n=38	n=38 2n=76	n=38 2n=76	n=19 2n=38
Other accounts	1. Wilkinson (1934) 2. Petrova, <i>et al.</i> (2007) 3. Barcaccia, <i>et al</i> (2003) 4. Mahmoud (1983) 5. Drušković (1995)		1. Takayuki Azuma (1999) 2. Bowden (1945)	Wilkinson (1944)	
3n=57					Ngantcha (2010)
2n=38	1. Mahmoud (1983) 2. Drušković (1995)	Mahmoud (1983)		Hakansson (1955)	1. Thibault (1998) 2. Harrison (1924)

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به کارهیتانی داتای دهنکه هه لاله و کروموسوم کان بو جیا کردنه وهی نیوان جوره کانی بیهوک له ههریمی کردستانی عیراق.

پوخته

دهنکه هه لاله و کروموسوم به کارهاتن بو بهراورد کردن له نیوان جوره کانی بیهوک له ههریمی کوردستان عیراق. نهم توئینه وهیه ده ریخست کهوا دریزی توه کان، پانی دیواری دهره کی، لوجه کانی دهره ی به شیوهیه کی بهرجاو دهوریان نیه له جیا کردنه وهی جوره کانی نهم روه که. بهلام گروپه کانی Erdtman 1971, 1945 بو قه باره و شیوه، سه ره ای بوونی دهنکه هه لاله ی (Parasyncolpate) سیفاتی گرنگن بو جیا کردنه وهی جوره کان. ژماره ی کروموسوم تا که کانی دهنکه هه لاله ی خانه ی دایک ده گاته 38 بو جوره کانی *s. acmophylla s. purpurea*، له کاتیکدا، ژماره ی جوتی ده گاته 76 بو جوار هیندبوون بو جوره کانی *s. aegyptiaca*, *s. babylonica*, *s. alba*. به گویره ی دهره نهنجامی ژماره ی کروموسوم کان *s. acmophylla* نزیکاتی هیه له گهل جوری *s. alba*، به ناسانی لی جیاده کریتته وه به هو ی بوونی جیاوازی له ژماره ی کروموسوم کان.

استعملت بيانات حبوب اللقاح والكروموسومات في التمييز بين انواع جنس الصفصاف في منطقة كردستان العراق.

الملخص

استعملت صفات حبوب اللقاح والكروموسومات للمقارنة بين انواع الصفصاف النامي في منطقة كردستان العراق. لقد اظهر الاختبار ان اطوال احاديذ الحبوب، سمك الجدار الخارجي ، الزخرفة السطحية تسهم بشكل غير معنوي في الفصل بين الانواع. بينما تعد مجاميع Erdtman 1971,1945 للحجم والشكل اضافة الي وجود حبوب القاح Parasyncolpate صفات هامة للتمييز بين الانواع. ان العدد الكروموسومات الاحادي لحبوب لقاح الخلايا الامية يبلغ 38 لانواع *S. aegyptiaca* , *S. purpurea* , *S. acmophylla* ، بينما يبلغ العدد الثنائي 76 للمتضاعفات الرباعية للانواع *S. acmophylla* , *S. alba* , *S. babylonica* طبقا لنتائج حساب العدد الروموسومي فان *S. acmophylla* القريب الصلة بالنوع *S. alba* لسهل التمييز عنه بسبب الاختلاف في العدد الكروموسومي.