USE OF WOOD MACERATED ELEMENTS FOR CLASSIFICATION OF *SALIX* L. SPECIES IN KURDISTAN REGION- IRAQ

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Summary:

Characters of wood macerated elements were investigated for taxonomic application. Wood samples from 5 stems of each *Salix acmophylla, S. alba, S. babylonica, S. aegyptiaca,* and *S. Purpurea* were collected from different geographical regions of kurdistan to clasp most possible variation. Wood of most species contains vessel elements described of primitiveness together with those descriptive of more or less advanced. Vessels of different species are similar in possessing the simple perforation plate. The differences are huge in vessel length and vessel width and hence in length/width ratio. Differences are also huge in vessel type or the end wall characters of vessel elements described of primitiveness and advancement, occurrence or absence of spiral thickening, cell walls pitting intensity and distribution within and between species. All these features are found to be important for providing taxonomic application. Libriform fibers are the only fibers present in the wood of this genus, no fiber trachieds occur. All species have fibers possessing dentition, almost exclusively at the distal parts, very rarely situated throughout the fiber length. Dentition morphological structure, distribution, size and orientation form important diagnostic characters.

Key words: Vessel elements, Libriform fibers, *Salix acmophylla*, *Salix Alba*, *Salix babylonica*, *Salix aegyptiaca*, *Salix purpurea*.

Introduction

There is about 526 species of *Salix* in the world (Fang-Zhen, 1987), most of which are distributed in the Northern hemisphere with only a few species in the Southern hemisphere. In Asia, according to the same researcher, there are about 375 species, making up 71.29 percent of the total *Salix* species in the world with the center of origin and development in the region between 20-40 º N in East Asia. In Iraq, 6 species are described, they are *S. alba*, *S. acophylla* Boiss., *S. babylonica* L., *S. purpurea* L., *S. aegyptiaca* L., *S. triandra* L. All of Iraqi representative species occur in Kurdistan region with the exception of *S. triandra* which has a very limited area of distribution at Tigris river banks near Rashidia north of Mosul city. Salix species are moisture demanding plants, commonly found in the lower and middle forest zones, in the latitudinal range 439-1840m (Abdulrahman, 2011), at river banks, moist mountain valleys, in riparian thickets, occasional in the moist steppe region. Willow species *S. alba* and *S. aegyptiaca* and *S. babylonica* need high soil moisture content and lower mean annual temperature compared to similar requirements for *S. acmophylla* and *S. purpurea*. In Kurdistan region, the species *S. acmophylla* is of especially high drought tolerance (Townsend and Guest, 1980). All willow species with the exception of *S. Babylonia* is found in the wilderness. Both *S. alba* and *S. acmophylla* are abundant and common in the region, while *S. purpurea* and *S. Aegyptiaca* are rare and occasional, especially in the western and central mountain sectors, they may be absent from the eastern sector. Only male plants of *S. purpurea* have been observed in Iraq.

According to Stace (1981) anatomical features are of particular value to scientists who need to identify small scraps of plant material. Some recent scientific works relate characteristics of the secondary xylem of *Salix* species to environmental conditions and pollution in Canada and Eastern Europe (Cooper and Cass 2001; Chavchavadze *et al.* 2002; Sizonenko and Chavchavadze 2002). On the other hand, Wagner (2009) studied the wood anatomy of young and adult samples of *Salix* × *rubens*, aiming to improve the knowledge about the most used species for basketry in South Brazil by relating wood anatomy characters of xylem quantity and ratio of pith/xylem to wood flexibility.

The vessel elements that originated in a precursor to the angiosperms may have been subsequently lost in some lineages like Amborellaceae, Tetracentraceae, Trochodendraceae, and Winteraceae. These

basal lineages were described by Cronquist (1981) as primitively vesselless. Moreover Cronquist considered the vessels of *Gnetum* to be converging with those of angiosperms. When data of vessel element characters of density, vessel lumen diameter, vessel length, and vessel clustering, in four endemic *Salix* taxa from the Lake Athabasca-Canada were compared by Cooper and Cass (2001) with the putative sister species for each endemic, it revealed similar vessel element densities to their associated sister species. In addition to that, the vessel element lumen diameter and length were significantly different in some of the species pairs. The authors indicated that the structural differences for these endemic willows appear to be related to their open sand habitat. The fiber dimorphism noticed in *Dubautia* and many other genera in which there are bands of thinner-walled fibers at intervals within a background of thick-walled fibers are probably a division of labor between fibers serving for photosynthate storage and fibers serving for mechanical strength (Sherwin, 1988). According to Sherwin (2014), most authors contrast wider, thinner-walled, shorter fibers with narrower, thicker-walled fibers that have narrower Lumina. For the same author, the evolutionary significance of fiber dimorphism in the form of few small changes in fiber structure can result in the achievement of different functions. Moreover, Sherwin (1984) indicated that the simple slit-like pits of libriform fibers cause minimal loss in mechanical strength compared to bordered pits.

Many researchers consider *Salix* as one of the most difficult genus for identification; there is still disagreement regarding the identity, number and distribution of species (Heywood *et al*. 2007; Mabberly 2008). The easy process of hybridization between species in the wild, together with their phenetic plasticity and different time of flowers and leaves development, made it difficult to observe all of these relevant characters on a single plant or specimen (Salih, T. *et al*. 2014). In many instances morphological characters are not sufficient to delimit closely related species of *Salix*. Therefore, in addition to morphological features, anatomical characters may provide valuable information for the classification of *Salix* (Arihan and Güvenç 2011).

The taxonomic relationships among the *Salix* species are not well understood and are still under debate. It is always felt for this genus that wood macerated elements should be viewed carefully in an attempt to provide useful taxonomic traits to identify taxa and demonstrate taxonomic relationships between species representative of Kurdistan-Iraq.

Material and Methods

Wood samples from *Salix acmophylla*, *S. alba*, *S. babylonica*, *S. aegyptiaca*, and *S. Purpurea* were collected from different geographical regions to clasp most possible variation. Samples of 2-3mm thick and 2-4cm long were prepared from growth increments 4-5, starting from the pith of the 5stems of each species, to avoid juvenile wood of it. The herbarium vouchers of them were deposited in the Biology department/Faculty of Science/University of Zakho.

Wood samples were macerated using Franklin solution (Franklin 1946). A sample of fibers was removed from the maceration and spread over a glass slide. 25 measurements of each of the following parameters were surveyed:

1. Libriform fiber length in mm.
2. Libriform fiber diameter (at the midpoint) in µm.
3. Libriform fiber wall thickness in µm.
4. Vessel element length in mm.
5. Vessel element diameter (at the midpoint) in µm.

Results and Discussion

Characteristics of the Genus:

Vessel element and libriform fiber dimensions are given in table (1). The overall range of vessel length and vessel diameter is found to be 0.2-0.55mm and 95.2-209µm respectively. These values together with STDs indicate high variance in vessel dimensions.

The vessel elements have simple perforation plates, inter-vessel pits are bordered pits, wood of most species contain vessel elements described of primitiveness together with those descriptive of more or less advanced.

Libriform fibers are the only fibers present in the wood of this genus, no fiber trachieds are to be observed. Fibers range 0.55- 1.20mm in length, 13.2-30.8µm in diameter at the midpoint, 1.5-7.0µm in cell wall thickness; statistics given in table (1) indicate low variability in measured parameters of the fiber. All species have fibers possessing dentitions, almost exclusively at the distal parts, vary rarely situated throughout the fiber length.

S. alba

Fibers of *S. alba* seems rather homogenous, mostly straight, thin-walled with small slit-like pits. Only distal dentitions occur on one side of some fibers, in most cases the dentitions of one end differ from dentitions of the other end, few fibers are with very smooth walls, containing no dentitions. Actually the overall fiber characters refer to a considerable modification and evolutionary process figure (1).

Vessels of *S. alba* exhibit high variability in shape and size. The wood tissue contains numerous vessel types at different level of advancement. According to Ghose (1984) and Klotz (1978) the vessel elements with simple perforation plate, horizontal end wall and least value of vessel length/vessel width are advanced, while those of scalariform perforation plate, sloping end wall and high vessel length/vessel width value are primitive. Considering these criteria, it is apparent that vessel type of *S. Alba* lay between the two extremes, none of them is more advanced or more primitive.

Number of lateral inter-vessel pitting areas differs from vessel to vessel, regardless of the level of advancement or primitiveness; it is a characteristic feature of this species.

Table (1): Mean, Standard Deviation (STD) and Range for Libriform Fibers and Vessel Elements.

Characters		*S . alba*	*S . acmophylla*	*S . babylonica*	*S . aegyptiaca*	*S . purpurea*
Libriform fiber length (mm)	Mean	1.01	0.93	1.10	0.88	0.90
	STD	± 0.11	± 0.14	± 0.12	± 0.1	± 0.13
	Range	0.77-1.19	0.66-1.15	0.8-1.2	0.69-1.04	0.55-1.1
Libriform fiber diameter (*μ*m)	Mean	20.93	19.70	23.22	20.8	20.2
	STD	±2.96	± 2.90	±3.11	± 2.35	± 2.93
	Range	15.4-25.3	13.2-24.2	19.5-30.8	16.5-24.0	15.2-26.0
Libriform fiber wall thickness (*μ*m)	Mean	3.4	4.2	4.1	3.1	4.3
	STD	± 0.93	± 1.08	± 1.35	± 0.80	± 1.45
	Range	2.2-4.4	1.5-6.0	2.0-5.3	2.1-4.1	1.8-7.0
Vessel element length (mm)	Mean	0.32	0.38	0.40	0.37	0.31
	STD	± 0.09	± 0.07	± 0.06	± 0.11	± 0.07
	Range	0.22-0.50	0.26-0.52	0.27-0.49	0.26-0.57	0.20-0.55
Vessel element diameter (*μ*m)	Mean	135.9	143.6	140.4	142.9	153.7
	STD	± 16.27	± 21.85	± 18.5	± 31.35	± 26.4
	Range	115.5-187	110-180.5	99-165	95.2-220	*108-209*

Each value represents 25 replicate

S. acmophylla

The fibers of this species express a high range of variation in fiber length and fiber cell wall thickness (table 1). Wide differences occur in fiber overall shape, some of them are quite straight; others are winding, especially at their distal parts (figure 2). Differences also occur in dental number, shape and distribution; mostly concentrated at the two fiber ends while some fiber possesses dentition distributed along the fiber length, forming a significant diagnostic character. Like *S. alba*, dentitions occur only on one side of some fibers, moreover dentitions of one end often differ from the dentition of the other end. Fiber wall is relatively thin, but thicker than that of *S. alba*, with small slit-like pits.

As figure (2) shows, vessel members of *S. acmophylla* are highly variable. End wall slopes of vessel elements show a wide range of variations such as, both end walls transverse, both end walls oblique and mixed type, i.e., one end wall transverse and the other oblique, the length/width ratio differences are also high indicating differential evolutionary process. The same figure refers to vessel shapes of short and transverse end wall possessing apomorphous character states in common together with members exhibiting pliesiomorphous character states of both end walls oblique. Moreover, mixed vessels of one end wall transverse and the other oblique are also found in the mixture of the macerated elements.

S. babylonica

The fiber length and fiber diameter measurements of this species are found higher with moderately thick cell wall compared to other *Salix* species (table 1). Fibers are ranging between straight forms to winding or slightly curved or zigzag with highly tapering distal ends. Dentitions differ in shape, number and size. Only distal fiber ends have dentitions, middle portions possess none of them. Number of dentitions are usually few, often one of them is conspicuous, either oriented upward to the fiber tip or perpendicular to the surface (figure 3), it is worth mentioning that the presence of such distinct dentition provide a significant taxonomic application. Pits, like other species, are small slit-like.

Vessel members macerated from the wood of this species show a great resemblance to one another, only two types of vessels are distinct; one possessing transverse and oblique end wall, while the other has oblique ends similar to those of very primitive vessel members, but not to the foraminate which contains several round perforations. These vessel members have lateral inter-vessel pits, mostly located around the perforation plate.

S. aegyptiaca

Fiber length and the fiber cell wall thickness are inferior to the corresponding measurements of other species. Fibers are rather irregular in shape; few seem to be straight with no or with very small dentitions. One of the distinct diagnostic character is the sudden reduction in the fiber diameter at both ends (figure 4) leaving three portions, two of them are strongly tapering distal portions and one constituting the median portion, the two tapering distal part may or may not equal the mid portion.

Statistics given in table (1) refer to high variability in length and diameter of vessel members and the occurrence of the high rate of large sized vessel in the macerated mixture. Vessel shapes displayed in (figure 4) shows different evolutionary levels (Ghose and Das, 2001)**,** ranging from most developed or advanced to the most primitive, but with higher ratios for the longest primitive members in the wood mixture. All vessel types of (i) both the end walls transverse (often typically quadrate) (ii) both the end walls oblique and (iii) mixed type are present. The occurrence of minute inter-vessel pitting and spiral thickening is a useful tool for identifying this species.

S. purpurea

The average fiber cell walls of this species are significantly thicker than cell wall of other species. Few fibers are found to exhibit straight forms; most are zigzag, curved or winding. Dentitions are restricted to the distal ends, ranging in number from 1to 6, on one side of the fiber. The two ends are often dissimilar in shape, number and size of dentitions. One of the diagnostic characters is the presence of course irregular of up to 6 dentitions on only one side of some fibers (figure 5).

The average length of vessel elements of *S. purpurea* is significantly lower than that of other species, but their average vessel diameter is significantly higher than other species (table 1). The advanced vessel type of both end walls transverse is common, one end wall transverse

and the other oblique is also common, while both the end walls with very slightly oblique is less common. The lower length/width ratio and the increased number of both end walls transverse are evidence of advancement of the wood tissue of this species more than any other *Salix* species.

From comparing different species, it reveals great differences in intensity of inter-vessel pitting; some members are even without lateral pitting. The former type of vessels which appear to be quadratic in cross section are very abundant, while the latter is rather rare and appear circular in cross section, a structure probably indicating the trend of wood tissue evolution. For the first type of the one end truncate and the other oblique, the length/ width ratio is highly variable probably referring to the different environmental adaptation. According to Haarer (1952) the result may be influenced by parental gene tree and other environment condition effect.

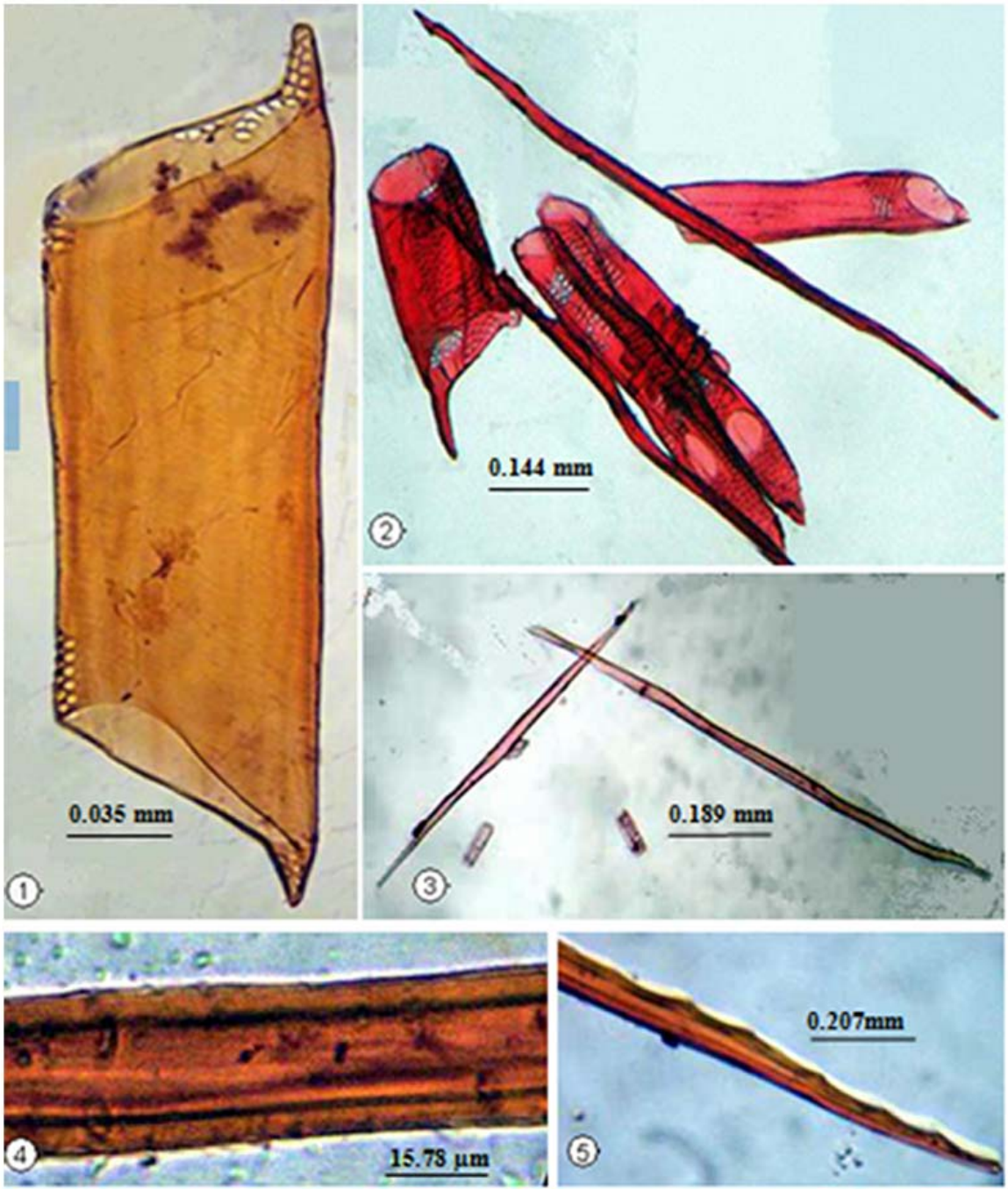

Figure (1): *S. alba*. 1. Vessel element, both ends slightly oblique, inter-vessel near adjoining vessels, 2. Vessels of variable ends and areas of pitting, 3. libriform fibers, 4. Medium part of a libriform fiber showing cell wall thickness and cell lumen, 5. Distal part of a libriform fiber showing dentitions on one side of the fiber.

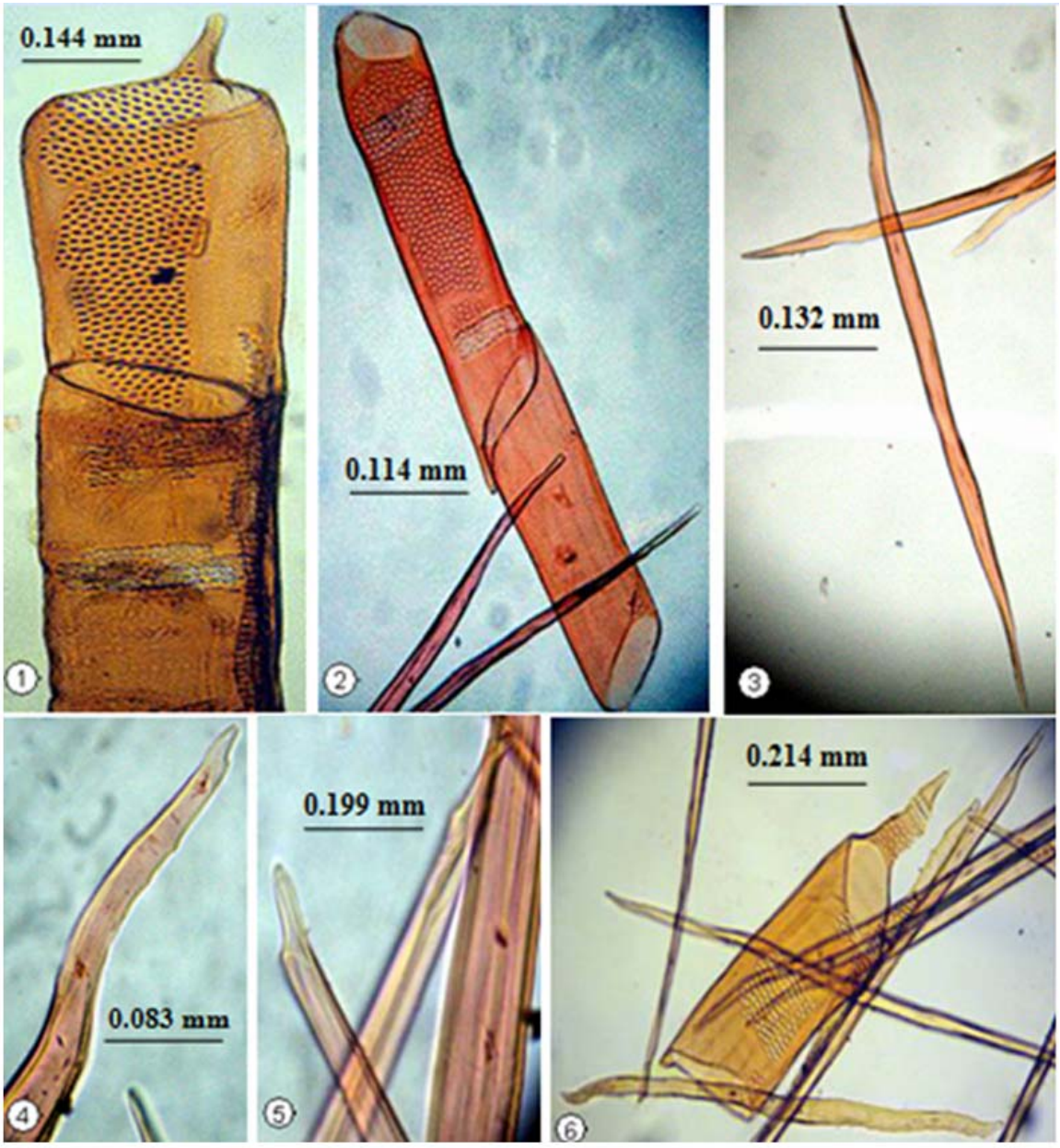

Figure (2): *S. acmophylla*: 1. Two ends by end connected vessel elements, both with two end transverse, 2. Two ends by end connected vessel elements, both with two ends oblique (140x), 3. Typically, straight-shaped libriform fiber, 4. Distal part of a fiber showing minute irregular dentitions, 5. Fiber cell wall and lumen with a fiber tip, 6. An irregular-shaped libriform fiber (below), Vessel member and numerous variously shaped libriform fibers (above).

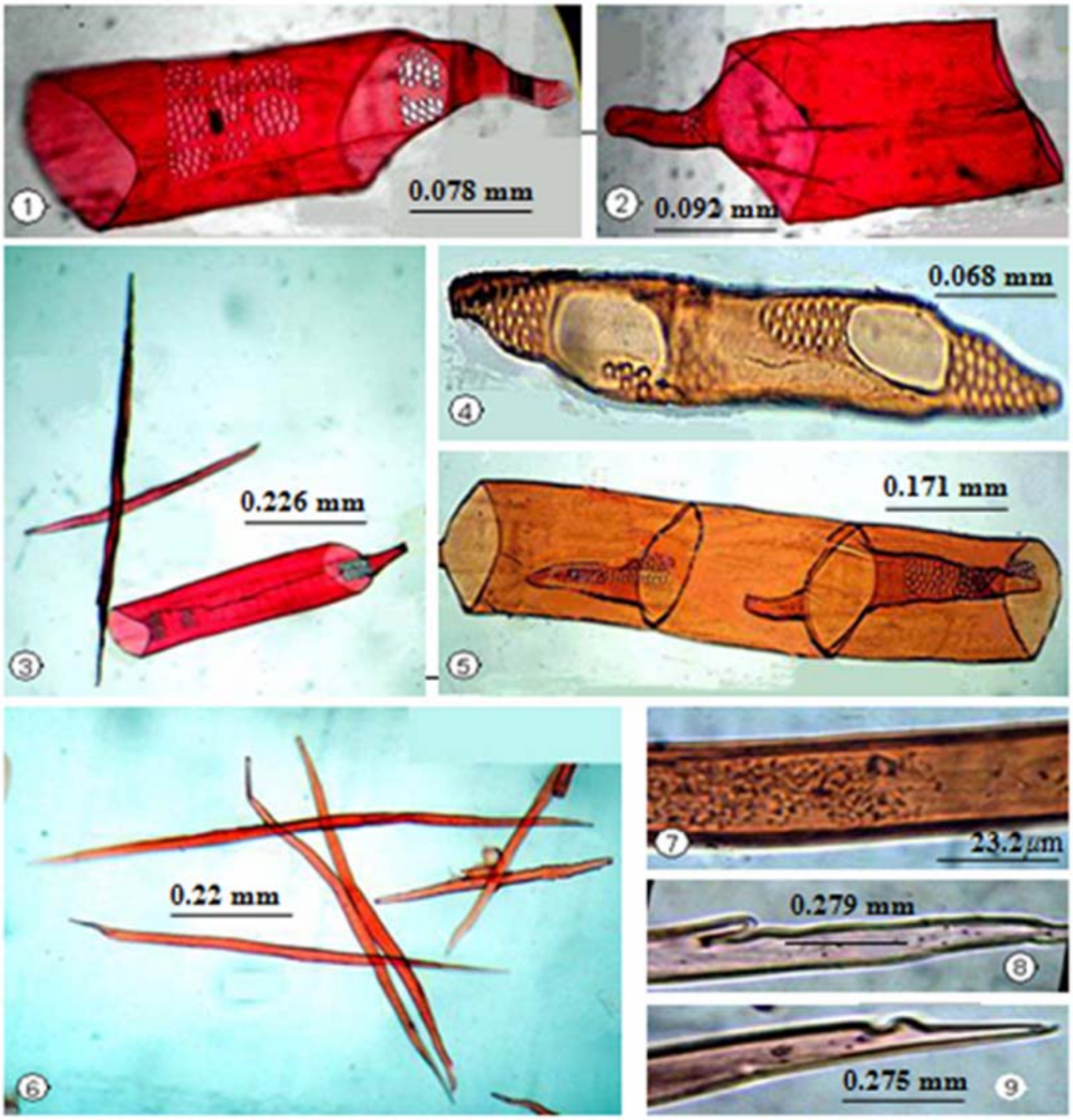

Figure (3): *S. babylonica*. 1. Vessel element of mixed end walls with intensive lateral pitting, 2. A vessel element of mixed end walls with no lateral pitting, 3. High length/width vessel element with libriform fibers, 4. A primitive type of a vessel element with both ends steeply oblique, 5. Two ends by end connected vessel elements, both with two end transverse, 6. Libriforms of different length and shapes, 7. Fiber cell wall thickness and lumen, 8 and 9. Distal fiber portions showing variously oriented dentitions.

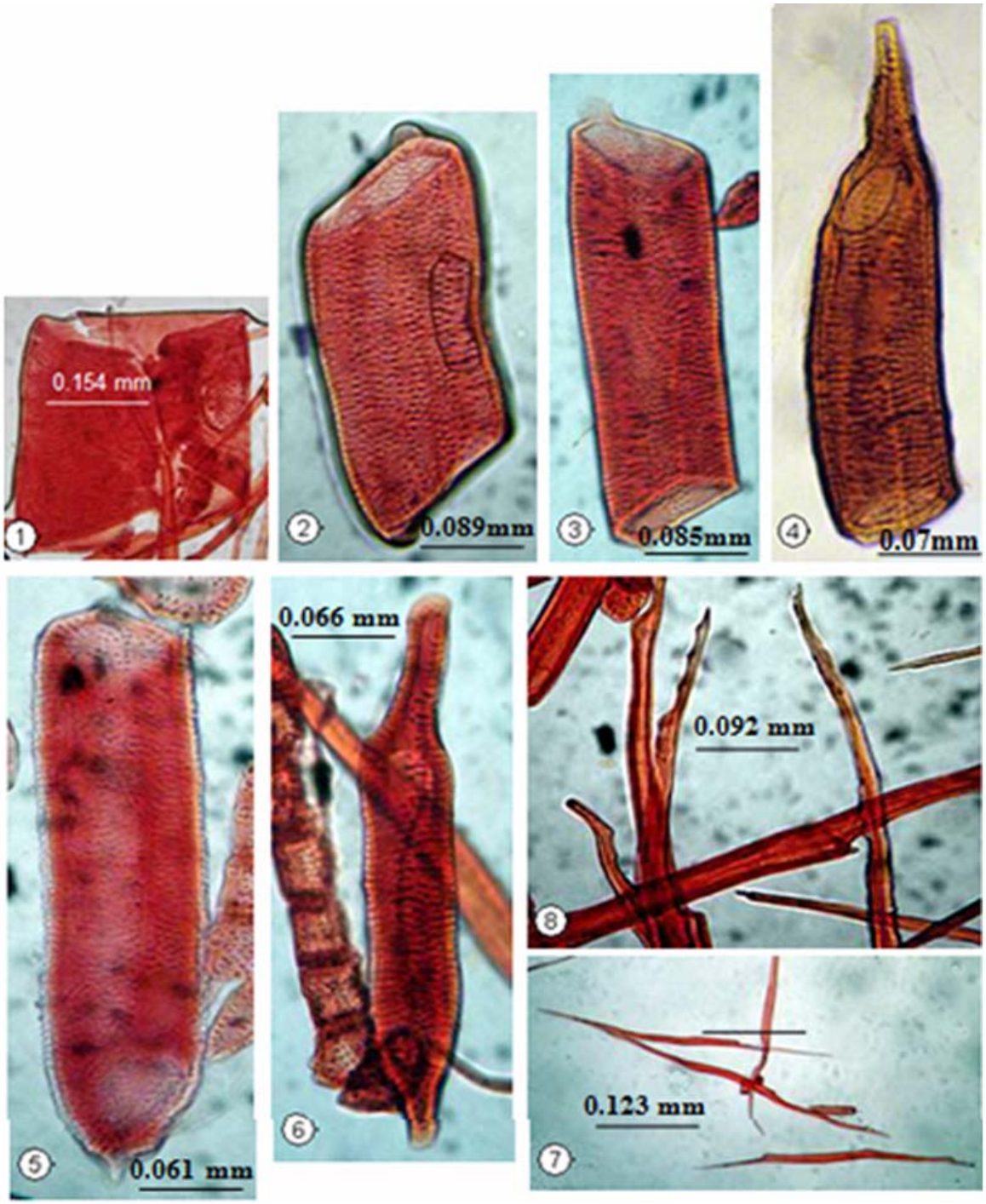

Figure (4): *S. aegyptiaca*. 1. Vessel member, very short with both ends transverse, 2 and 3. Vessel members of more or less transverse end walls, 4. Vessel member with one end wall transverse and the other oblique, 5. Vessel member with both ends oblique, 6. A very primitive vessel member with the both end walls steeply sloped, 7. Different types of libriform fibers, 8. Distal fiber portions showing different types of dentitions.

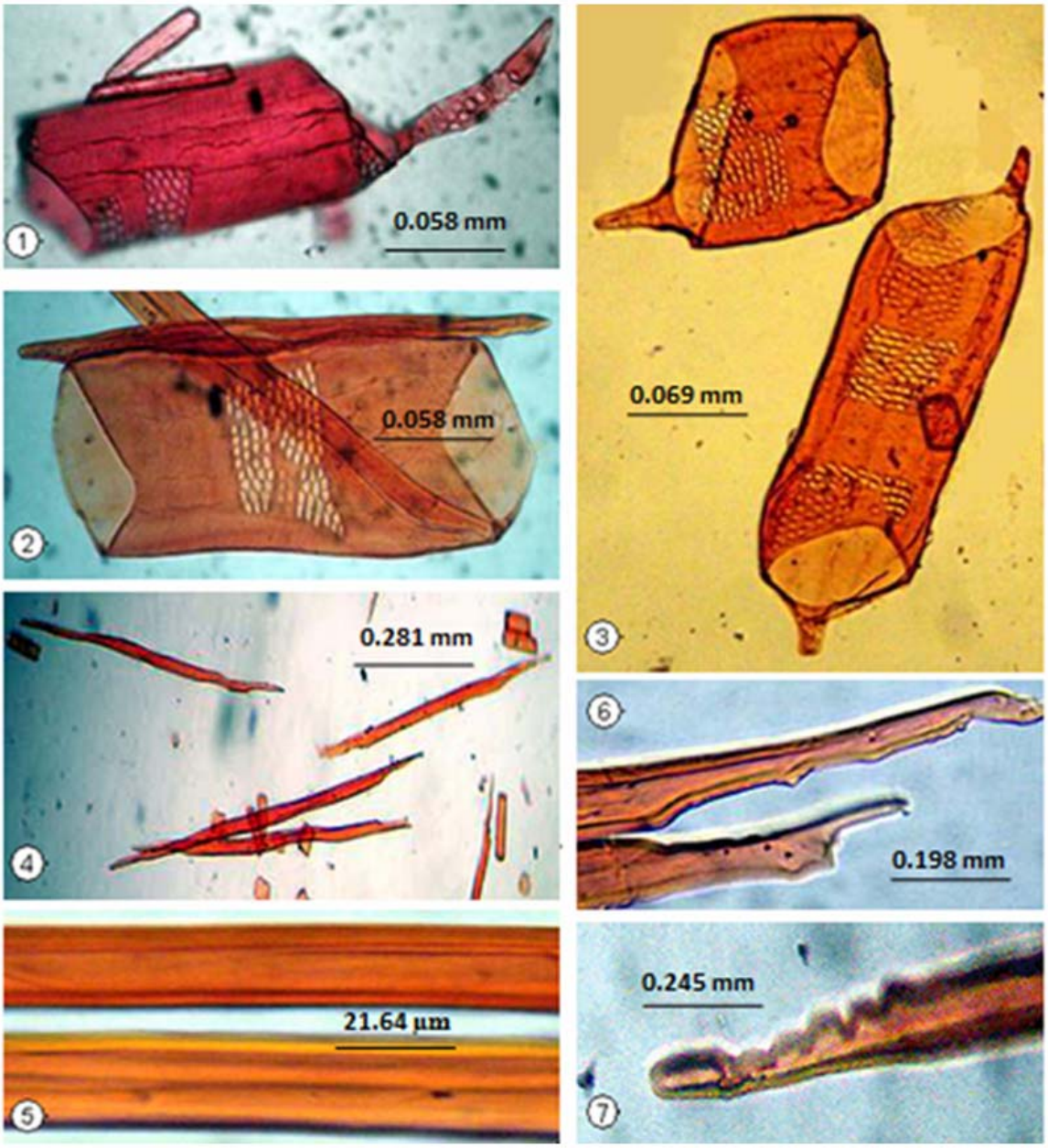

Figure (5): *S. purpurea*. 1. Vessel member with one end oblique and the other transverse, 2. Vessel member both end walls transverse, 3. Two vessel members, one (above) withmixed end walls, the other (below) with both end walls slightly oblique, 4. Libriform fibers, 5. Fiber cell wall thickness and cell lumen, 6 and 7. Two types of distal portions showing variable kinds of dentitions.

Important taxonomic applications:

A. **Fiber dentition:**

1. Dentitions of most fibers occur only on the distal ends: *S. alba, S. babylonica, S. aegyptiaca, S. purpurea*.

2. Dentitions of some fibers occur along the length of the fiber: *S. acmophylla*.

B. **Dentition size and orientation:**

1. One of dentitions on the distal portion is large and conspicuous, either oriented upward to the fiber tip or perpendicular to the surface: *S. babylonica*.

2. None of the dentitions are so large and conspicuous, little differences occur between them: *S. alba, S. acmophylla, **S. aegyptiaca**, S. purpurea*

C. **Fiber structure:**

1. Sudden reduction in the fiber diameter at both ends, dividing the fiber into three portions, two at both ends, one median: *S. aegyptiaca*.

2. Gradual reduction in the fiber diameter towards ends: *S. alba, S. acmophylla,* S. *babylonica, S. purpurea*.

D. **Vessel length:**

1. Vessel element never exceeds 0.55mm in length: *S. alba, S. acmophylla, S. babylonica, S. purpurea*.

2. Vessel element exceeds 0.55mm and reaching 0.57mm in length: *S. aegyptiaca*.

E. **Vessel morphology:**

1. Vessels with minute inter-vessel pitting and spiral thickening: *S. aegyptiaca*.

2. Vessels with larger-sized inter-vessel pitting, with no spiral thickening; *S. alba, S. acmophylla, S. babylonica, S. purpurea*.

F. **Vessel type**:

1. Wood macerated mixture containing vessel type with both the end walls steeply oblique: *S. babylonica, S. aegyptiaca*.

2. Wood macerated mixture containing no vessel type of both end walls steeply oblique: *S. alba, S. acmophylla, S. purpurea*.

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بکارئینانا پیکهاتێن دارکێ رووەکێ جودا جودا ژ بو مەرەما پولێن کرنا جوری رووەکێ (صفصاف) ل هەرێما کوردستانا عیراقی...

کورتی:

هاتە بکارئینانا ساخلەتێن پیکهاتێن دارکێ رووەکێ جودا جودا بو تاقیکرنا بنەماییێن پولین کرنی.ژ هەرجورەکی ژڤان رووەکا(Salix acmophylla,S.babylonica,S.aegyptiaca,S.purpurea,S.alba)

پێنج نموونێن چەقی دارکێ هاتینە وەرگرتن. ئەڤ نموونە هاتێنە وەرگرتن ژ جهێن جیاواز بو دیارکرنا بلندترێن ئاستی جیاوازیا ڤی جوری رووەکێ.دەرئەنجامی ڤی لیکولینی دا هاتیە دیارکرن زوربەی ڤان جۆرین رووەکا پیکهاتێن وی سەرەتاییە ئەگەر ئەم بەراورد بکەێن دگەل وان جۆرین رووەکا ئەوی پیکهاتێن وی تا رادەیەکێ ئالوز.لوولی هەمی جورا وەکێ ئیکە ژ هەبینا هێلەکە بۆریەکان سادە بەلێ هاتیە دیارکرن تەنها جیاوازیەکا مەزن یا هەی ژ لایێ دریژیێ و پانیا لوولەیێ ڤە.

لسەر وی چەندیێ را هاتیە دیارکرن کو چەندێن جیاوازییێن مەزن ێت هەێن ژ لایێ شیوازی لوولەیێ ڤە و ساخلەتین دیواری ڤە یێ دوماهیکا پیکهاتێن لوولەکیێ.هاتیە ئاماژەکرن ب سادەو پەرەسەندی و هەبینا و نەبینا ستوورىونێن لوولپیچی و هەروەسا چری و بلاڤە بینا کونەکان.دیاربی ئەڤ هەمیێ ساخلەتێن مە دیار کرین گرنگیەکا مەزن یا هەی ژ بو بکارئینانا بنەماییێن پولێن کرنیێ.دارکێ ڤی جۆری رووەکێ دا تەنها جورەکی ژ ریشالا هاتینە دیتن کو دبیژنێ (لایریفورم).بەلێ جهێ ئاماژیە کو ریشالێن نیانیێ دا ئەڤ جوریێ ریشالا نەهاتێە دیتن.سەری ڤان جورە ریشالا هندەك شیوازێن ددانا یێن هەێن ئەڤ شیوازی ددانا تەنها لسەری ریشالا دا یێ هەی.بەلێ نەهاتیە لسەر دریژاهیا ڤیێ ریشالیێ.شیوازیێ پیك هاتنا ڤان ددانا وبلاڤبوونا و قەبارە و ئاراستەییێ وان لسەر دریژیا ریشالیێ گرنگیەکا مەزن یا هەی ژ لایێ پولێن کرنیێ

استعمال العناصر الخشبيه المنفصله فى تصنيف انواع جنس الصفصاف *Salix L.* في منطقه كردستان العراق .

الملخص:

**درست صفصاف العناصر الخشبيه المفصولة لأجل التطبيقات التصنيفيه اذا جمعت العينات من خمسة سيقان لكل من أنواع التالية لنبات الصفصاف , (*Salix acmophylla, S.alba, S.purpurea, S.aegyptiaca ,S.babylonica)*
والتى اختيرت لتمثل مناطق جغرافية مختلفة للحصول على اعلى تباين ممكن. اثبتت الدراسة بأن خشب معظم الانواع يضم العناصر الخشبية البدائية فضلا عن تلك التى توصف بالاقل او الاكثر تقدما .بينت النتائج تشابه مختلف الانواع فى امتلاكها صفيحة مثقبة بسيطة فضلا عن وجود اختلافات كبيرة فى طول وعرض الاوعية وبالتالي نسبة الطول /العرض وقد لوحظ ايضا وجود اختلافات كبيرة فى نوع الوعاء الخشب وصفات الجدران النهائية لعناصر الاوعية البدائية منها فضلا عن توزيع وغزارة النقر ضمن النوع الواحد وبين الانواع المختلفة وتعد جميع الصفات اعلاه ذات اهمية فى التطبيقات التصنيفية . الالياف الطويلة ذات النهايات الضيقة هي الوحيدة التى وجدت ضمن هذا الجنس، فصلا عن وجود الالياف القصيبية كما وجد بأن جمع الانواع قد اظهرت تسننات وبوجه خاص فى نهايات البعيدة ونادرا ماظهرت على طول الليف يشكل التركيب الشكلي لتسننات، توزيعها ، حجمها واتجاهها صفات تشخيصية هامة.**