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## Morphological, Molecular and Pollen Grain Investigations of *Salix* Species in Egypt

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### Abstract:

Genus *Salix* is among family Salicaceae, distributing in the northern hemisphere. It is represented in Egypt by two species (*Salix mucronata* and *Salix tetrasperma*). The classification of *Salix* at the generic and infra-generic levels is still outstanding. We have agreed to list the Egyptian species of this genus. We collected them during field trips to most Egyptian habitats; fresh and herbarium specimens were subjected to taxonomic revision based on morphological characters; scanning electron microscope (SEM) for pollen grains; isozyme analysis using esterase and peroxidase enzymes and genetic diversity using random amplified polymorphic DNA (RAPD). We recorded that both sexes of *S. mucronata* existed but there were only male trees from *S. tetrasperma*. SEM of pollen grain revealed that there were many differences between them. Also, the application of isozyme analysis giving 12 sex indicating bands. In addition, ten RAPD primers retrieved a total of 227 amplified bands (77 for female *S. mucronata*, 74 for male *S. mucronata* and 76 for male *S. tetrasperma*).

**Key words:** Egypt, Isozyme, Pollen grain, RAPD, *Salix*.

### Introduction:

Genus *Salix* Linnaeus (1753: 1015) of Salicaceae, is known as willows commonly (1). It is moisture loving trees and shrubs, with long lanceolated leaves, it is planted around banks to hold soil in suburban yards (2). It comprises ca. 350–520 species, distributed mainly in the northern hemisphere, absent in Oceania, except the introduced species (3). The systematics taxonomy and of *Salix* have proven extremely difficult because of their simple flowers, dioecious reproduction, common natural hybridization, and large intraspecific phenotypic variation (1). On the other hand, (2) revealed that the classification of *Salix* at the generic and infrageneric levels is still unresolved; also, the essential taxonomic difficulties of the genus *Salix* are too increased by their high

phenotypic plasticity and great variation in diagnostic morphological characters

In Egypt it is represented by two species; *Salix tetrasperma* Roxb., *Salix safsaf* Trautv. (Latter treated as *Salix mucronata* Thunb.). It is distributed in Nile and Faiyum regions, in addition to Mediterranean coastal strip and eastern desert, also, both species are represented in western desert Oases (4).

In plant taxonomy, the best evidence in the flowering plants, especially in the angiosperms is the application of pollen morphology (5). Also (6) mentioned that pollen morphological characters were useful in the investigation of the Asteraceae family, as well as that of some of its genera and species. Pollen grain in angiosperm is clearly divisible into two different types: heteropolar,

bilateral, boat-shaped monosulcate pollen versus isopolar, radio-symmetric, globose tricolpate pollen (7). Wodehouse RP 1937 (8) revealed that the pollen grain of *Salix* is tricolpate and heavily reticulate, on the other hand (9) cited that the pollen grain of *Salix* usually 3-colporoidate (sometimes 2-colporoidate), subprolate or more frequently prolate.

Random Amplification Polymorphic DNA (RAPD) is a PCR-based technique introduced by (10). RAPD markers technique can be used in conservation genetics, population genetics, genetic diversity among crop species, and in taxonomical classification studies (11). Markers relationships provided a quick genetic marker for the study of species in addition (12) revealed that the use of these markers is a cost-effective, fast, highly discriminative and reliable technique. RAPD marker has been used for determining sex in *Salix viminalis*, *Pistacia vera*, *Atriplex garrettii* and *Trichosanthes dioica* (13). The inter- and intra-specific relationships within *Salix* species in Egypt can be studied using RAPD markers.

The isozyme is the basic analytical tool to study the molecular mechanism. Also, (2) revealed that isozymes and allozymes are among the biochemical markers with a paramount importance where they have been the most important type of genetic marker used for many

different applications in many species. As an important genetic marker, it could be used to detect the inheritance and variance among different species or different cultivars at the molecular level. It also could be as a biochemical marker to study the tolerance of plant to stress (14).

Till now the classification of *Salix* at the generic and infrageneric levels is still unresolved (2). Because molecular markers have so many advantages over morphological markers, we decided to classify the Egyptian species of this genus using RAPD and isozymes markers in addition to pollen grain characters.

### Material and Methods:

We collected 30 samples (11 male *S. tetrasperma*, one male *S. mucronata* and 18 female *S. mucronata*) from various localities across the country during spring 2007 and 2008 (Fig. 1), and specimens were checked at Egyptian herbaria. The lists of species recorded were identified according to (15),(16), (4).

The fresh and herbarium specimens were subjected to taxonomic revision based on macro- and micro-morphological characters. The macro-morphological description is mainly based on field and laboratory studies with reference to (4).



Figure 1. Map of Egypt showing the distribution of the studied genus *Salix* collected during this study.

### Laboratory analysis

Macro-morphological characters cited in this work are based on the earlier works of (16),(4) and the current specimens descriptions.

Micro-morphological characters including pollen grains investigation, isozyme and RAPD analysis have been carried out as follows:

#### Pollen grain

Pollen grains of the *Salix* species under investigation were obtained from the collected samples. Materials were prepared for SEM by mounting acetolyzed pollen grain on clean stubs using double sided cello tape, coated with gold and examined by a JOEL JSM 5400 LV scanning electron microscope operated at accelerated voltage of 15 kv, at Electron Microscope Unite, Assiut University. The description of pollen grains has been demonstrated with the literatures as (17)

#### Isozymes

Juvenile leaves of *Salix* species under investigation have been collected during March from the field. Leaves have been kept in -20°C for isozyme studying.

Esterase and peroxidase (PRX) have been assayed using 100 mg freshly harvested leaves of the studied species according to the method of (14).

#### DNA isolation

DNA samples have been extracted from 50 mg of juvenile leaf tissues in each sample; according to (18). Sequences of the used primers are outlined in Table 1.

**Table 1. Primers used for RAPD analysis**

Primer code	Sequence (5' - 3')
OPA-05	AGGGGTCTTG
OPA-07	GAAACGGGTG
OPA-18	AGGTGACCGT
OPA-20	GTTGCGATCC
OPD-01	ACCGCGAAGG
OPD-04	TCTGGTGAGG
OPO-02	ACG TAG CGT C
OPO-14	AGC ATG GCT C
OPP-01	CCTCTGCCCA
OPP-16	CCAAGCTGCC

### Results:

#### Taxonomic results

Specimens' examination (fresh and herbarium) revealed that genus *Salix* in Egypt is represented by two wild species (*S. mucronata* and *S. tetrasperma*). This work clarifies that female *S. mucronata* Thunb trees are distributed along Nile valley and delta and extends eastward to Ismailia in the eastern desert, while the few male *S. mucronata* Thunb trees were traced only in Fayium region. On the other hand, *S. tetrasperma* Roxb has been traced

only as male trees but female trees are never found in Egypt. This species is distributed in Nile delta and northern part of the Nile valley, to 28° N, and not traced in the southern part.

#### Morphological features

*Salix mucronata* Thunb., Prodr. P1. Cap. 6(1794).

The plant is shrub or tree up to 8 m, deciduous, bark brownish. Leaves 12 x 2 cm (female plant) 16.3 x 3.2 cm (male plant), lanceolate, serrate, acute, pale lower surface. Juvenile leaves covered with simple hairs. Petiole 0.8 -1.5 cm in female plant, 0.3 - 2.1 cm in male one. Stipules persistent, ovate, serrulate, acute. Female inflorescence catkin 1.5 -3.1 x 0.3 - 0.7 cm, yellow to dark brown, peduncle to 0.4 cm. Bract elliptic, entire, acuminate. Flower 2 .0 - 2.5 mm, yellow. Bract sagitate, entire, acute, hairy of simple hairs. Ovary 0.1 - 0.9 x 0.4 - 0.6 mm, yellow, glabrous. Style 0.2 - 0.4 mm, brown. Stigma 0.1-0.3 mm, brown. Capsule 5.0 - 6.0 mm, ovoid. Male inflorescence catkin 3.9 - 4.5 x 0.8 -1.0 cm, whitish yellow. Peduncle 0.3 - 0.5 cm. Bract lanceolate, serrulate, acute. Floral bract 6.0 -7.0 x 3.0 -3.5 cm, ovate, entire, acute, covered by simple hairs. Stamen 6 - 8, anther 1.0 -1.3 mm, filament hairy lower half as shown in Fig. 2.



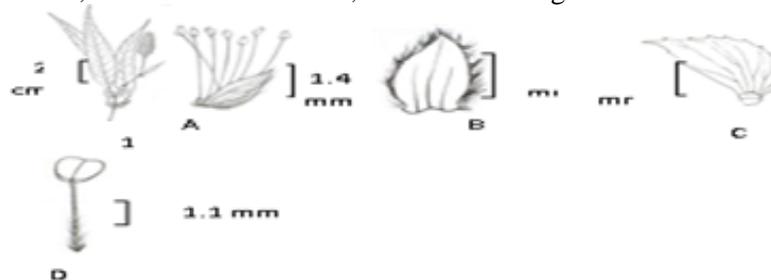
**Figure 2. *Salix mucronata*; 1 and 2 branches with mature leaves and inflorescence to female and male plant; respectively. A, female and male flowers; B, floral bract; C, stipule; D(2), stamen.**

*Salix tetrasperma* Roxb., P1. Coast Corom. 1:66, t.97 (1798)

Shrub or tree is up to 12 m, deciduous, and it is sometimes evergreen, dark brown. Leaves to 18 x 6 cm, in Egypt leaves 18(-25) x 6(-7.7) cm, broadly ovate-lanceolate, serrate, acuminate, pale lower surface, juvenile leaves are covered with simple hairs. Petiole 0.4 - 1.6 cm. Stipules persistent, ovate, serrulate, acute. Male inflorescence catkin, in Egypt it measures (2.5 -) 8 (-11) x 0.5-1.0 cm, pale yellow. Peduncle to 0.7 cm.

Bract lanceolate, entire, acuminate. Flower 2.0- - 2.6 mm, yellow. Perianth 1.1 – 1.2 mm long, 0.2 – 0.3 mm wide, entire, acute, covered with simple unicellular hairs. Stamens 6, anther 1.0 x 1.3 mm,

filament hairy lower half. Female flower in catkin 8 – 12 cm; ovary sessile, hairy; fruit pyriform; female trees less common in Egypt than male trees as shown in Fig. 3.



**Figure 3.** *Salix tetrasperma*; 1, branch with mature leaves and inflorescence; A, flower; B, floral bract; C, stipule; D, stamen (female plant not traced in Egypt).

### Key to the *Salix* species

leaves more than 6.0 cm diameter; inflorescence up to 11.0 cm long. ....male *S. tetrasperma*

leaves not exceeding 3.5 cm diameter; inflorescence not exceeding 4.5 cm long ..... male *S. mucronata*

leaves not exceeding 2 cm diameter; inflorescence not exceeding 3.1 cm long ..... female *S. mucronata*

### Pollen grains of *Salix* spp.:

The result of pollen grains investigations as seen by SEM are outlined in Table 2. The pollen grain shape of the studied *Salix* species is radiosymmetric, elliptical and tricolpate (Fig. 4C and D). The pollen grain size is outlined in Table 2, denoting that the largest pollen grain is that of *S. tetrasperma* (E x P: 13.33 x 15.50  $\mu$ m). While the smallest pollen grain is of *S. mucronata* (E x P: 11.51 x 19.31  $\mu$ m).

The data, outlined in Table 2, demonstrate that the colpus length of the studied *Salix* species can be used as differential character between them. The colpus length in *S. mucronata* not exceeding 10  $\mu$ m (Table 2, Fig. 4B). While the colpus length of *S.*

*tetrasperma* (Fig. 4E) is nearly equal (up to 16.50  $\mu$ m; Table 2).

Pollen of the studied *Salix* species shows a dimorphism character, this means that the presence of two types of each lumen width in pollen grain. The reticulum dimorphism of the studied species appears clearly *S. tetrasperma* where it is gradually reduced towards colpei while in *S. mucronata* the lumen width has the same size all over the pollen grain (Fig. 4B and E).

The network lumen of the studied *Salix* species shows clearly distinguishable pattern. This pattern can be summarized as follows: the network lumen in case of *S. tetrasperma* exceeds 1.0  $\mu$ m, while this is not applicable to *S. mucronata*. *S. tetrasperma* possesses the widest network lumen (1.15 x 0.85  $\mu$ m; Fig. 4F), while that of *S. mucronata* is the smallest lumen (0.55 x 0.30  $\mu$ m; Fig. 4C).

The exine sculpture of the lumen in *S. tetrasperma* appears granulate (Fig. 5F), while in *S. mucronata* is a psilolumena (Fig. 4C).

Muri of the studied species is irregular and undulate form its thickness do not exceed 1.0  $\mu$ m, in the two species studied (Table 2, Fig. 4C and F).

**Table 2. morphometric characteristics of the *S. mucronate* and *S. tetrasperma*.**

<i>Salix</i> species	E ( $\mu$ m)	P ( $\mu$ m)	E/P	Colpus LxW ( $\mu$ m)	Lumen LxW ( $\mu$ m)	Muri ( $\mu$ m)
<i>mucronata</i>	10.80–12.22	17.50–21.11	0.09	7.69 – 11.78 x 0.35 – 0.76	0.20- 0.90 x 0.10 – 0.50	0.14- 0.60
	(11.51)	(19.31)		(9.73 x 0.55)	(0.55 x 0.30)	(0.37)
<i>tetrasperma</i>	12.00–14.66	15.00–16.00	0.03	13.30 – 17.00 x 0.60 – 1.50	0.20- 2.10 x 0.20 – 1.50	0.27- 0.90
	(13.33)	(15.50)		(15.15 x 1.05)	(1.15 x 0.85)	(0.58)

E: equatorial diameter

P: polar axis

Mean value between brackets.

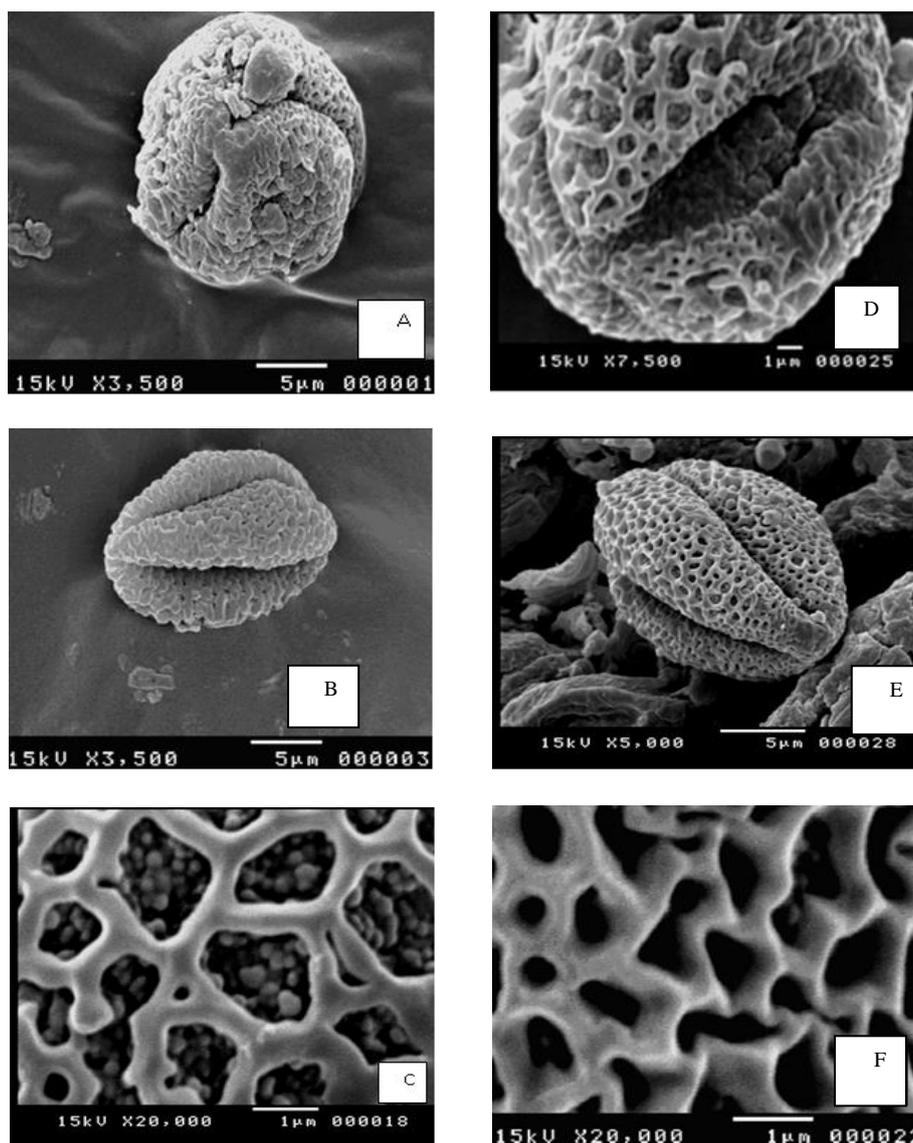


Figure 4. SEM micrograph of pollen grain of the studied species *S. mucronata* and *S. tetrasperma*, A & D. polar view, B & E. equatorial view, C & F. exine sculpture.

#### Isozyme analysis:

The zymograms for Esterase and Peroxidase are outlined in Fig. 5A and B.

Esterase: Nine esterase bands were developed in the studied *Salix* species: namely  $\alpha$ ,  $\beta$  and  $\alpha/\beta$  esterase. Where  $\beta$ 3 is a common esterase band in the studied male and female of *S. mucronata* specimens.  $\alpha$ 4 esterase band developed only in male of *S. tetrasperma* specimens.  $\beta$ 2 band present in all

the *Salix* specimens studied, in all localities and it appeared as a sex non –related isozyme (Table 3, Fig. 5A).

Peroxidase (PRX): The developed PRX zymogram showed three bands. The band 3 at  $R_f = 0.85$  appeared only in female *S. mucronata*. On the other hand, the band1 at  $R_f = 0.27$  presents in all studied specimens (Table 3, Fig. 5B).

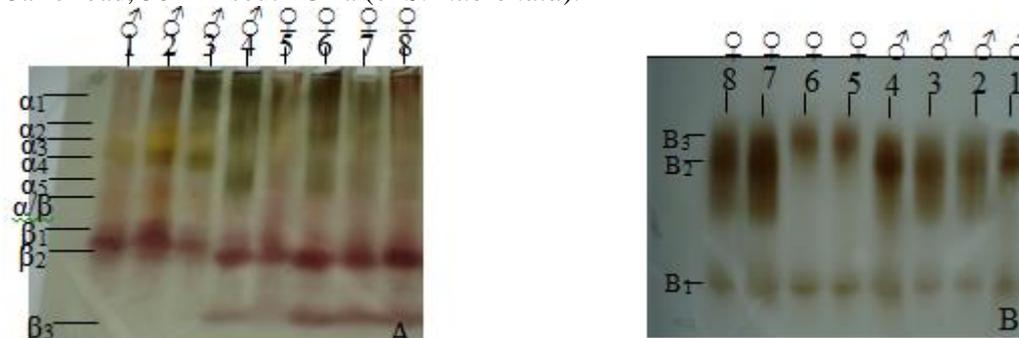
**Table 3. The Pattern of Esterase and Peroxidase isozymes in *S. mucronata* and *S. tetrasperma*.**

Taxa	RF	<i>S. tetrasperma</i>			<i>S. mucronata</i>				
Sample no.		1	2	3	4	5	6	7	8
Isozyme		♂	♂	♂	♂	♀	♀	♀	♀
<b>Esterase</b>									
$\alpha 1$	0.06	++	+	-	+++	+	+++	+	++
$\alpha 2$	0.2	+	-	-	++	-	+++	+	++
$\alpha 3$	0.23	+	+	+	-	+	-	-	-
$\alpha 4$	0.25	++	+++	+	-	-	-	-	-
$\alpha 5$	0.3	-	-	-	+++	-	+++	+	+
$\alpha/\beta$	0.43	-	++	+	-	-	-	-	-
$\beta 1$	0.49	-	+++	++	-	-	-	-	-
$\beta 2$	0.56	++	+++	+++	+++	+++	+++	++	+++
$\beta 3$	0.72	-	-	-	++	+	++	++	++
<b>Peroxidase</b>									
Band 1	0.27	+	+	+	+	+	+	+	+
Band 2	0.77	++	++	+++	+++	-	-	+++	+++
Band 3	0.85	-	-	-	-	++	++	-	-

(-): absence of band.

(+): presence of band (increasing in number of + sign means increasing in strength of band).

1:♂ Ismailia, Abou Dahshan village 2:♂ Kafr El-Sheikh; 3:♂ Alex. Cultivated road, Tokh (of *S. tetrasperma*).  
4:♂ Beni-Suef-El Fayium road, 22 Km east El Faiyoum; 5:♀ Beni-suef-El Fayium road, 14 Km west Beni-Suef; 6:♀ Draw-Aswn road, 10 Km south Draw; 7:♀ Beni-Suef-El Fayium road, 12Km west Beni-Suef; 8:♀ Beni-Suef-Cairo road, 50 Km south Giza (of *S. mucronata*).



**Figure 5. Zymograms of the studied *Salix* specimens: A: Esterase and B: Peroxidase. Specimens 1-3: *S. tetrasperma* and 4-8: *S. mucronata*.**

**RAPD analysis:**

RAPD results outlined in Fig.6 A,B and C displayed three examples of the polymorphism between *Salix* species using ten positive primers ( OPP-16, OPD-01, OPP-01, OPA-07, OPA-20, OPO-14, OPA-05, OPA-18, OPD-04 and oPo-02; Table 4).

The number of scorable bands per primer varied between 8 as in primers OPD-04 & OPO-02, (Fig. 6B) and 16 as in primer OPO-14, (Fig. 6C), giving a total of 227 bands. Out of these bands, there are 37 bands showed unimorphism in both *Salix* species and presence of 23 bands characterized *S. tetrasperma* from *S. mucronata*

(Table 4, Fig. 6 A, B and C). Out of the ten screened RAPD primers, only eight primers (OPD-01, OPP-01, OPA-07, OPA-20, OPO-14, OPA-05, OPA-18 and OPD-04) showed polymorphism between male and female *Salix*. OPD-01 produced 1300 bp band, and OPA-05 produced 830bp, 800bp and 620bp bands in male *S. mucronata* only. On the other hand, female *S. mucronata* showed a large number of the developed bands in seven primers among these bands are: bands at 900bp and 870bp developed by OPP-01 primer and bands at 800, 700 and 650bp developed by OPA-07.

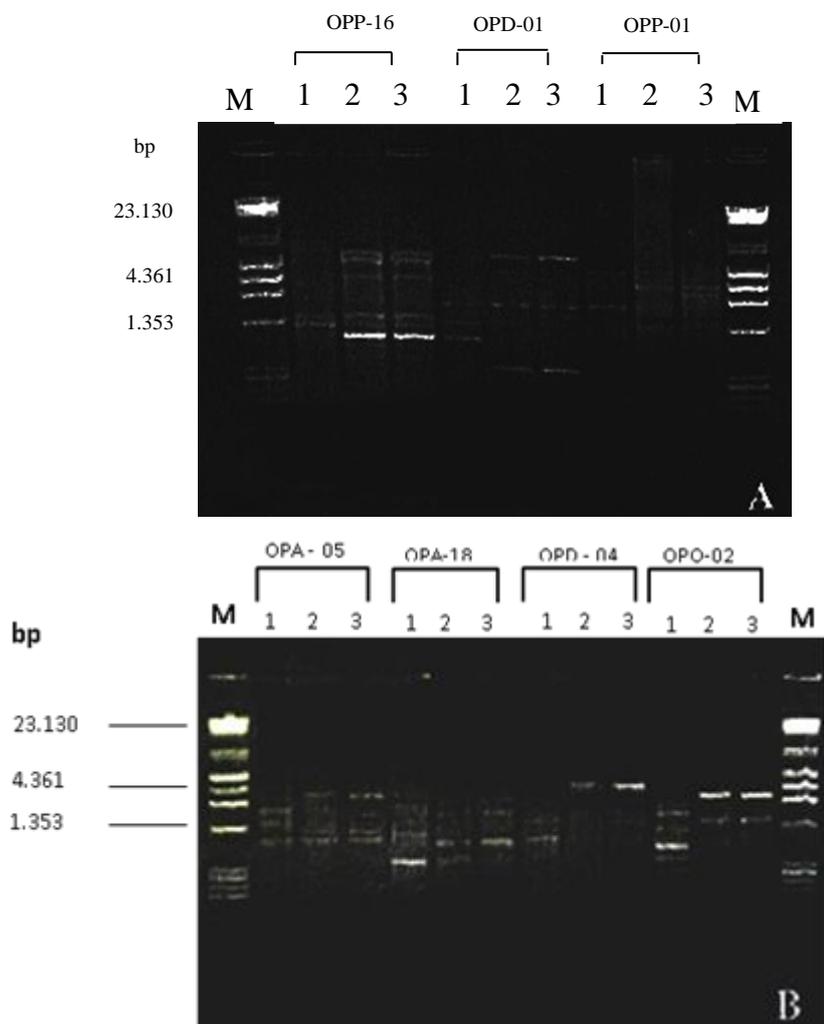
On the other hand, number of bands is differentiated between male *S. mucronata* and *S.*

*tetrasperma*. like 2000bp and 650bp bands amplified by the OPP-16, 600bp and 550bp bands appeared in OPD-01, 2000bp also, 800bp, 700bp, 570bp and 520bp bands amplified by the OPO-02 primer from the genomic DNA of all the male *S. tetrasperma* entries was absent in the PCR products of the DNAs from all the male *S. mucronata* entries.

In addition, there were six primers (OPA - 07, OPA - 20, OPO-14, OPA - 05, OPA- 18 and OPD- 04) that give bands difference between male (*S. mucronata* and *S. tetrasperma*) and female (*S. mucronata*) as shown in Table 4.

**Table 4. Ten RAPD primers and their respective bands in (*S. mucronata* and *S. tetrasperma*)**

Code	Amplified bands			Unique bands			Polymorphic bands	Sex determined bands
	♀ <i>S. mucronata</i>	♂ <i>S. mucronata</i>	♂ <i>S. tetrasperma</i>	♀ <i>S. mucronata</i>	♂ <i>S. mucronata</i>	♂ <i>S. tetrasperma</i>		
OPP-16	8	8	7	-	-	2	5	-
OPD-01	6	7	6	-	1	2	4	-
OPP-01	12	10	9	2	-	2	7	-
OPA-07	7	7	9	3	1	3	2	5
OPA-20	12	9	8	2	-	1	6	2
OPO-14	9	7	12	3	-	4	3	6
OPA-05	4	7	8	-	-	3	2	3
OPA-18	10	10	6	1	-	-	5	2
OPD-04	5	5	5	1	-	2	2	2
OPO-02	4	4	6	-	-	4	2	-
<b>Total</b>	<b>77</b>	<b>74</b>	<b>76</b>	<b>12</b>	<b>2</b>	<b>23</b>	<b>38</b>	<b>20</b>



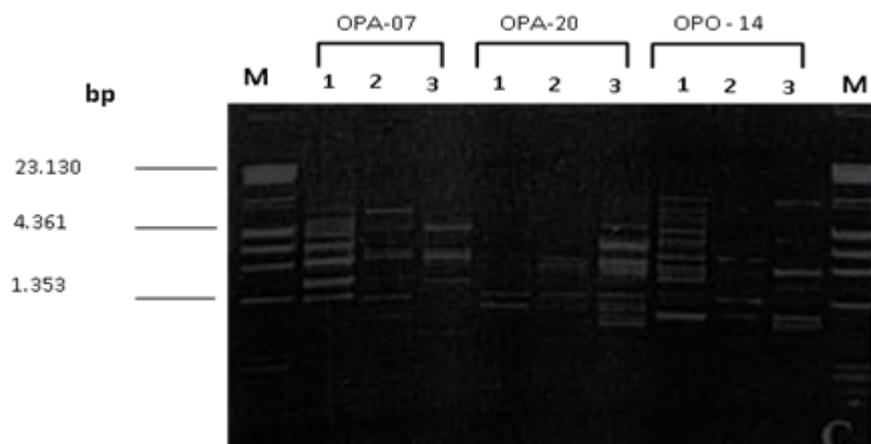


Figure 6. A, B and C: RABD pattern of the *Salix* species studied using ten primers: 1 ♂ *S. tetrasperma*; 2 ♂ *S. mucronata*; 3 ♀ *S. mucronata*

### Discussion:

Genus *Salix* in Egypt is represented by two wild species (*S. mucronata* and *S. tetrasperma*). This result is supported by earlier studies of (4,16). Leaves of *S. mucronata* is narrower than that of *S. tetrasperma* which is supported by (4,17).

The esterase and peroxidase patterns showed variability in the anodal region among all tested specimens. The appearance of these bands may be due to 1. The presence of several genes controls these isozymes. 2. Their allelic states (homozygous or heterozygous). 3. Quaternary structure of the protein products; and 4. Their subcellular compartmentalization (19).

The developed zymograms showed the presence of characteristic bands ( $\beta_2$  in esterase, Band 1 in peroxidase) to *Salix* species. Esterase and peroxidase revealed the presence of sex – differential bands among them:  $\alpha_2$ ,  $\alpha_3$  and  $\alpha_5$ -esterase and Band 2 & Band 3 in case of peroxidase. These bands appeared to be sex – linked bands. On the other hand, there are other sex – bands that are not differential among them  $\alpha_1$ ,  $\beta_2$  and  $\beta_3$  esterases and Band 1 peroxidase. Based on earlier data, esterase and peroxidase enzymes can be used for sex identification such as (20) where he claimed that in many plant species, the aspect of PRX has been used to distinguish males and females in dioecious plants, also (21) proposed that peroxidases could be exploited as a marker for sex identification in high diversity in date palm inflorescence..

The SEM investigations, carried out on the Egyptian *Salix* species, revealed a tricolpate, reticulate elliptical grain. This is supported by (8) who claimed that the Pollen grains of *Salix* are tricolpate and heavily reticulate.

The results of the present study showed that the two studied *Salix* species can be distinguished from each other by several characters. *S. tetrasperma* has the largest pollen grain and widest exine lumen with granulate lumen sculpture. While *S. mucronata* has the shortest colpus, smallest lumen and psilolumina lumen sculpture. This is supported by (22) where they reported that the width of lumina and muri was considered as one of the characters by which *Malcolmia*, *Strigosella* and *Zuvanda* species could be distinguished.

The studied *Salix* species possess primitive characters, such as reticulate exine. This postulation is supported by earlier study like, (23), where he proposed that family *Salicaceae* is a primitive dicotyledons, and occupy a very lowly position in Engler system. Also, tricolpate is another primitive feature of the studied *Salix* pollen grain which was confirmed by (24), they also claimed that monosulcate and tricolpate, basic types of aperture in angiosperm are represented as ancestral forms to the most advanced types.

Despite these primitive characters, the *Salix* pollen studied shows some advanced characters. This was established earlier by (25), who mentioned that morphology of genus *Salix* has some primitive characters and other advanced ones. Among the observed advanced characters are: radiosymetry of the pollen studied. This denoting that *Salicaceae* was derived from ancestor family possesses elongate bilateral symmetry pollen. This postulation was supported by (7), where they demonstrated that the radiosymetry and isopolar characters are relatively advanced characters evolved from primitive types through intermediate stage. The second relatively advanced character is the elliptic pollen shape. This result was supported by (7), where they claimed that elliptic is a relatively

advanced character derived from boat-shaped monosulcate pollen. The present investigation denoted both primitive and advanced characters are represented in the studied species, where *S. mucronata* has the smallest grain size and the coarseness exine reticulum. On the other hand, *S. tetrasperma* has the largest grain size and the coarsest exine reticulum. This result was confirmed by (26) where they claimed that large pollen grain size with coarse reticulate exine in *Eucharis* (Amaryllidaceae) is considered as a primitive character.

Wodehouse RP 1937(8) mentioned that in pollen grain of genus *Salix*, the mesh of the reticulum is generally coarse toward the centers of the lunes, finer toward the poles, and sharply bounded along the margins of the furrows.

Knowledge of genetic relationships among genotypes is useful in any plant breeding program. In *Salix*, there is currently limited information on genetic diversity and genetic relationships within and between species, clones and hybrids in the gene pool (27).

Within the limits of male and female genotypes available in this study, the RAPD bands OPP-01900, OPP-01870, OPA-07800, OPA-07650, OPA-07350, OPA-201300, OPA-201100, OPO-141200, OPO-14700, OPO-14500, OPA-18300 and OPD-041300 appeared to be the female sex-related DNA marker in *S. mucronata*. On the other hand the RAPD bands OPA-071800, OPA-071350, OPA-07600, OPO-14900, OPO-14800, OPO-14600, OPA-05830, OPA-05800, OPA-05620, OPA-18870 and OPD-04550 clearly distinct to be male sex-related DNA marker in two studied *Salix* species. These results are supported by (28) who reported that the RAPD bands used were linked to a sex-determining locus in family Salicaceae. On the other hand, the approach of RAPD-PCR technology has been successfully applied to identify the sex of *H. rhamnoides* (29); in addition, (30) used RAPD and SCAR analysis to differentiate between sexual forms of *Papaya*.

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### Authors' declaration:

- Conflicts of Interest: None.
- We hereby confirm that all the Figures and Tables in the manuscript are mine ours. Besides, the Figures and images, which are not mine ours,

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- Ethical Clearance: The project was approved by the local ethical committee in University of Beni-Suef.

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## دراسة مقارنة بين الأنواع التي تتبع جنس الصفصاف في مصر

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### الخلاصة:

جنس الصفصاف هو من العائلة الصفصافية منتشر في نصف الكرة الشمالي علي مستوي العالم. ممثل في مصر بنوعان (الصفصاف العريض والصفصاف الرفيع). حتى الان لا يزال تصنيف الصفصاف به مشاكل ولذلك هدف الدراسة هو تصنيف الأنواع المصرية من هذا الجنس. قمنا بجمعها خلال الرحلات الميدانية لمعظم البيئات المصرية. خضعت العينات الخضراء والمجففة لمراجعة تصنيفية على أساس السمات المورفولوجية، مسح مجهر الكتروني لحبوب اللقاح وتحليل الأيزوزيم باستخدام انزيمات الاستيراز والبيروكسيداز والتنوع الجيني باستخدام الحمض النووي المتعدد الأشكال العشوائي. سجلنا وجود كلا الجنسين من الصفصاف الرفيع ولكن لم يكن هناك سوي أشجار ذكور من الصفصاف العريض. كشفت حبوب اللقاح عن وجود اختلافات كثيرة بينها. أيضا تطبيق تحليل الأيزوزيم يعطي تشير 12 bands الي الجنس بالإضافة الي ذلك استعادت عشرة بادئات RAPD مجموع 227 نطاقات مكبرة (77 للإناث و 74 للذكور في الصفصاف الرفيع و 76 للذكور في الصفصاف العريض).

الكلمات المفتاحية: مصر، إيزوزيم، حبوب اللقاح، RAPD، *Salix*