



TAXONOMIC EVALUATION USING POLLEN GRAIN SCULPTURE AND SEED COAT CHARACTERS OF 11 TAXA OF GENUS *HIBISCUS* (MALVACEAE) IN EGYPT

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Keywords: Pollen grains morphology, *Hibiscus*, Seed coat, SDS-PAGE

ABSTRACT

Pollen grain morphology and seed coat characters of 11 cultivars belonging to two species of genus *Hibiscus* (Family Malvaceae) namely *H. esculentus* (*H. Abemoschus*) and *H. sabdariffa* were investigated. This study was carried out using light microscope (LM) and Scanning Electron Microscopy (SEM). Pollen morphology of this genus is fairly uniform. Generally radially symmetrical apolar, mostly spheroidal, pantoporate. Seed exomorphic characters revealed four types of ornamentations; reticulate, ocellate, foveolate and ruminant. Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE) was employed to characterize those taxa. Thirty one bands of seed protein profiles have been constructed from the gel. The produced dendrograms were analyzed by STATISCA program using UPGMA clustering method showed a close affinity among the seven *H. esculentus* cultivars and the four *H. sabdariffa* cultivars.

INTRODUCTION

The genus *Hibiscus* is widespread, it comprises about 200 annual and perennial species. Kenaf is closely related to cotton, okra, and the hollyhocks. Kenaf, along with roselle, is classified taxonomically in the *Fucaria* section of *Hibiscus*. This section includes between 40 and 50 species (distributed throughout the tropics) that are closely related morphologically (Dempsey, 1975). The genus is represented in the Egyptian flora by 5 species; most of them are in the Mediterranean coastal re-

gion. Characters illustrated from seeds as a protein and isozyme have been utilized in plant taxonomy at different hierarchical levels to construct the phenetic classification Abd El-Hady, (2007). Christensen, (1986) stated that the generic delimitation based on pollen morphology is difficult in this genus. El-Nagaar, (2004) studied the pollen morphological characters of 21 species of Egyptian Malvaceae belonging to 10 genera of this family. On the other hand, Tahavi, (2000) stated that pollen morphology of this family is fairly uniform. Perveen *et al* (1994) and Perveen & Qaiser (2007) reported that the present pollen data are based on pollen morphology of four species and three cultivars of the genus *Hibiscus*. Pollen morphology of four species and three cultivars belonging to genus *Hibiscus* (Malvaceae) from North West of Pakistan were examined under light and scanning electron microscope by Noreen *et al* (2008). Polyacrylamide Gel Electrophoresis for protein and isozyme was used for identification of species, subspecies and variety level (Adriane *et al* 1969 and Boulter *et al* 1970). The aim of this work is to provide complete information about seed coat morphology and pollen morphology of the genus *Hibiscus* growing wild and cultivated as ornamental plants in Egypt to characterize and resolve the extent of similarities and variations between different studied cultivars of *Hibiscus*.

MATERIALS AND METHODS

Seeds of *Hibiscus* L. cultivars were obtained from different localities in Egypt. Eleven accession belong to two species of genus *Hibiscus* L. was investigated and the sources of these taxa are given in Table (1).

Table 1. Species and cultivars locations and section of the studied taxa of *Hibiscus L.*

<i>Studied Species</i>	<i>Cultivar</i>	<i>Location</i>	<i>Section</i>
<i>H. esculentus L.</i>	Red	Fayum	Hibisceae
	Municipal	Menoufia	Hibisceae
	Musician	Assuit	Hibisceae
	Municipal	Giza	Hibisceae
	Red	Tanta	Hibisceae
	Ezabi without Bhuk	Banha	Hibisceae
	Ezabi Bhuk	Al-Qanater	Hibisceae
<i>H. sabdariffa L.</i>	Municipal	Bani Suefe	Furcaria
	Ezabi	Tanta	Furcaria
	Municipal	Hosh Issa	Furcaria
	Ezabi	Hosh Issa	Furcaria

I- SEM Techniques

Pollen grains were collected after cultivation in Egypt to obtain pollen grains of buds and stored in refrigerator at 3-5°C until used. Samples of the studied taxa were acetolyzed according to Erdtman's technique (Erdtman, 1952). Dried pollen grains were mounted onto clean stubs using double-slided adhesive, the samples were coated with a 30nm layer of gold using fine coat ion sputter JEOL-JFC-1100E ion-sputtering device at accelerated voltage of 15 Kv at the scanning electron microscope unit of Ain Shams University. The seeds of the studied taxa were washed thoroughly with distilled water to remove any impurities on their surfaces, dried and soaked in 10% HCl for 6 hours to remove the coat enveloping the seed (Ismail and El-Ghazaly, 1990). This was followed by washing the seeds with distilled water then dried and prepared for SEM examination by mounting these dry seeds onto clean stubs using double sided adhesive tapes. These clean dry seeds were coated with gold film in JEOL JFC 1100E ion- sputtering device. Then, the coated seeds were viewed and photographed with JOEL ISM-5500LV scanning electron microscope, which operated at accelerated voltage of 15 KV at the Scanning Electron Microscopy Unit in Al-Azhar University.

II- Electrophoretic Techniques

Extraction and analysis of seed protein fractions were carried out by using one dimensional sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE). Preparation and running of gel were carried out according to Stegmann *et al* (1980). The gel was stained with coomassie brilliant blue stain R- 250. Bands were determined and scanned by using Hoefer Scanning densitometer GS 300. Protein gel bands were scanned and photographed in (Plate III).

RESULTS AND DISCUSSION

Pollen grain morphology of *Hibiscus* under LM (light microscope) showed fairly diversity in the shape, size and aperture diameter; these characters are summarized in Table (2). Generally, the pollen grains of this genus were spherical with pantoporate shaped (Plate I). The largest pollen grain diameter 90.5 µm was noticed in *H. esculentus* cultivar *Municipal* (Giza), whereas the lowest one 80.50 µm was found in *H. esculentus* cultivar *Red* (Fayum). Also, the values of 86.50 µm and 85.5 µm were recorded in cultivar *Musician* (Assuit) and cultivar *Ezabi Bhuk* (Al-Qanater), respectively. In addition the values of 84.50 µm and 82.5 µm were recorded in cultivar *Municipal* (Menoufia) and cultivar *Red* (Tanta) [Plate (I) Figs. (1-7)]. On the other hand, the pollen grain diameter in

Table 2. Pollen grains morphological characters of the studied cultivars of *Hibiscus L.*

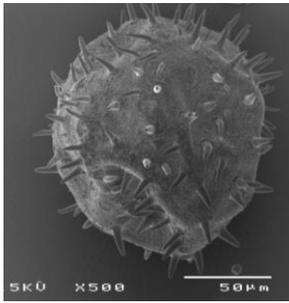
Characters Taxa	Pollen diameter (μm)	Exine thickness (μm)	Spine height (μm)	Spine Basal (μm)
<i>H. esculentus</i> cultivar Red (Fayum)	80.5	3.70	11.01	3.50
<i>H. esculentus</i> cultivar Municipal (Menoufia)	84.5	3.60	11.02	3.60
<i>H. esculentus</i> cultivar Musician (Assuit)	86.5	3.72	11.04	3.50
<i>H. esculentus</i> cultivar Municipal (Giza)	90.5	3.75	11.03	3.70
<i>H. esculentus</i> cultivar Red (Tanta)	82.5	3.72	11.02	3.75
<i>H. esculentus</i> cultivar Ezabi without Bhuk (Banha)	89.5	3.70	11.02	3.70
<i>H. esculentus</i> cultivar Ezabi Bhuk (Al-Qanater)	85.5	3.69	11.00	3.71
<i>H. sabdariffa</i> cultivar Municipal (Bani Suefe)	63.5	3.46	10.03	5.00
<i>H. sabdariffa</i> cultivar Ezabi (Tanta)	67.5	3.49	10.05	5.01
<i>H. sabdariffa</i> cultivar Municipal (Hosh Issa)	70.5	3.52	10.06	5.08
<i>H. sabdariffa</i> cultivar Ezabi (Hosh Issa)	76.5	3.56	10.07	5.09

H. sabdariffa cultivars ranged from 63.5 μm to 76.5 μm , where the highest value of 76.50 μm was determined in *H. sabdariffa* cultivar *Ezabi* (Hosh Issa), whereas the lowest one of 63.50 μm was found in *H. sabdariffa* cultivar *Municipal* (Bani Suefe). Also, the values of 70.50 μm and 67.50 μm were recorded in the taxa of cultivar *Municipal* (Hosh Issa) and cultivar *Ezabi* (Tanta) respectively [Plate I] Figs. (8-11)].

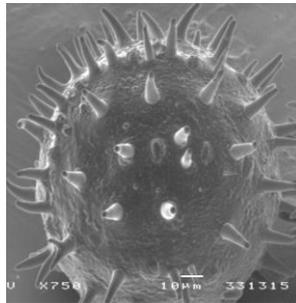
Seed exomorphic characters of 11 cultivars of genus *Hibiscus L.* characters are summarized in Table (3) showed variations at specific level, to some extent in seed size, shape and colour. The seed surface of all cultivars is characterized by smooth appearance under light microscopy, but such cultivars differ in their shapes and dimensions. Seed colour varied from black in the 7 cultivars of *Hibiscus esculentus*, pale red to dark red in the 4 cultivars of *Hibiscus sabdariffa*. The largest LW ratio of 1.42 was found in *H. esculentus* cultivar *Municipal* (Menoufia), whereas the lowest ratio of 1.09 was found in *H. sabdariffa* cultivar *Ezabi* (Tanta). The SEM revealed the presence of reticulate form with different small particles in cultivars of Fayum, Menoufia and Banha and showed ocellate on seed surface pattern with broad raised circular

borders in cultivars Assuit, reticulate form with dense wax materials on their surface (Tanta cultivar) and reticulate without wax materials (Al-Qanater). On the other hand, SEM revealed the presence of reticulate form in cultivars of cultivar *Ezabi* (Tanta), cultivar *Ezabi* (Hosh Issa) and *Municipal* (Hosh Issa). Also, the ruminant pattern was found in *H. sabdariffa* cultivar *Municipal* (Bani Suefe) respectively in (Plate II).

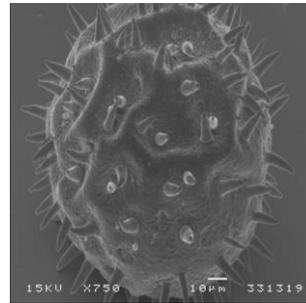
The dendrogram produced from the cluster analysis of the different cultivars of genus *Hibiscus* based on pollen grain and seed coat characters is represented in (Fig. 1). From the dendrogram; the *H. esculentus* cultivar *Ezabi* without *Bhuk* (Banha) was split in a single level at the dissimilarity level of 100 %. The remainders of the studied cultivars were divided into two groups; the first subgroup included the four cultivars of *H. sabdariffa* collected from (Bani Suefe, Tanta and Hosh Issa). In such subgroup, *H. sabdariffa* cultivar *Ezabi* was separated at distinct taxonomic level of 75%. The second subgroup included the remainder of the studied taxa of *H. esculentus* which collected from different localities (Fig. 2). In the second subgroup the cultivars of *H. esculentus* cultivar *Red* (Tanta) was split in a single level of 85%.



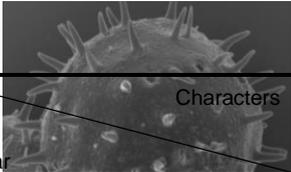
1- *H. esculentus* cultivar Red (Fayum)

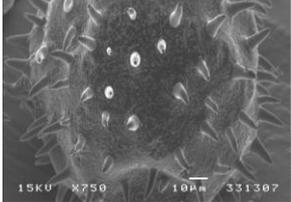


2- cultivar Municipal (Menoufia)

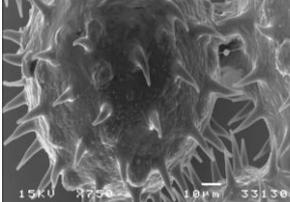


3- cultivar Musician (Assuit)

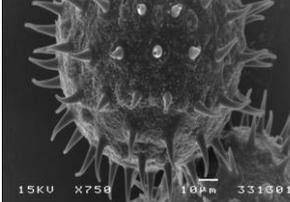
	Characters	Seed shape	Seed Length (L) (mm)	Seed Width (W) (mm)	L/W	SEM Observation
cultivar						
<i>H. esculentus</i> Red (Fayum)		ovate	4.70-4.75	3.90-4.00	1.20	reticulate
<i>H. esculentus</i> Municipal (Menoufia)		round	5.40-5.50	3.2-4.50	1.42	reticulate
<i>H. esculentus</i> Musician (Assuit)		depressed ovate	4.90-5.00	3.2-4.50	1.25	ocellate
<i>H. esculentus</i> Municipal (Giza)		ovate	5.40-5.50	4.90-5.00	1.10	foveolate



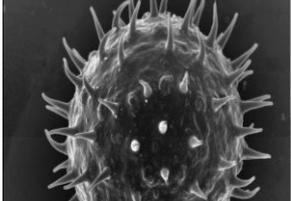
7- cultivar *Ezabi* Bhuk (Al-Qanater)



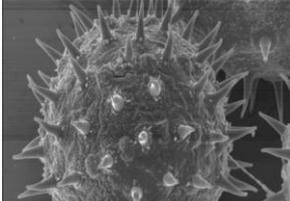
8- *H. sabdariffa* cultivar *Municipal* (Bani Suefe)



9- cultivar *Ezabi* (Tanta)



10- cultivar *Municipal* (Hosh Issa)



11- cultivar *Ezabi* (Hosh Issa)

Plate I. SEM photographs show pollen grain morphology of the studied accessions of genus *Hibiscus* L.

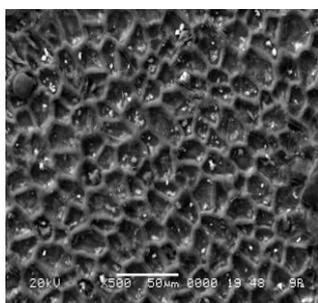
Table 3. Seed morphological characters of the studied taxa of the genus *Hibiscus* L.

<i>H. esculentus</i> Red (Tanta)	depressed ovate	4.90-5.00	3.90-4.00	1.25	reticulate
<i>H. esculentus</i> Ezabi without Bhuk (Banha)	round	4.90-5.00	3.20-4.50	1.29	reticulate
<i>H. esculentus</i> Ezabi with Bhuk (Al-Qanater)	ovate	4.90-5.00	3.20-4.50	1.29	reticulate
<i>H. sabdariffa</i> Municipal (Bani Suefe)	kidney	6.00-6.25	4.50-4.75	1.32	ruminant
<i>H. sabdariffa</i> Ezabi (Tanta)	kidney	5.20-5.50	4.80-5.00	1.09	reticulate
<i>H. sabdariffa</i> Municipal (Hosh Issa)	kidney	4.90-5.00	4.30-4.50	1.13	reticulate
<i>H. sabdariffa</i> Ezabi (Hosh Issa)	kidney	5.30-5.50	4.50-4.75	1.17	reticulate

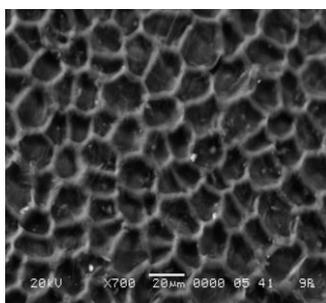
The dendrogram produced from the cluster analysis of the 11 cultivars of the genus *Hibiscus* based on seed protein profiles is represented in **Fig. (2)**. The studied cultivars were separated at taxonomic level of 100.0%, the cultivar 2 and cultivar 6 (Menoufia and Banha) were separated at a distinct taxonomic level of 92.0 %. In addition, cultivar Giza was separated in a single level of 99.0%. The remainders of the studied taxa were separated in two groups; the first included the studied cultivars of *H. esculentus* (Fayum, Assuit and Tanta). The second group included the studied taxa of the four cultivars of *H. sabdariffa*. Two sub groups have been recognized, the first one included the studied cultivars of 8 & 10, the second subgroup included the remainder of *H. sabdariffa* (**Fig. 2**).

It is obvious that pollen morphology of the different cultivars are fairly uniform, such results agree with the results of (**Tahavi 2000**). The captured images of the pollen grains by LM and SEM revealed that pollen grains of these two species are spherical-shaped with pantoporate with a great variation in their diameters and spinate form in

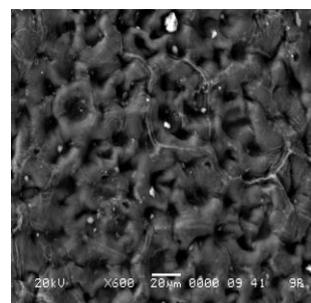
each taxa. The results obtained from the LM (diameters of pollen grains; pores number and pore diameters) and SEM (aperture numbers on the pollen grain surface) examination showed that there is a considerable diversity in pollen grains morphology of the cultivars of *Hibiscus* at the specific and intraspecific levels. Our results are in agreement with the results of **El-Naggar (2001 & 2004)** and (**Tahavi, 2000**). The pollen grain diameters of species belonging to section *Furcaria* (seven cultivars of *H. esculentus*) are larger than those of section *Hibisceae* (four cultivars of *H. sabdariffa*). However, pollen morphology will have limited use in species identification because of similarities across species. Also, our results are in accordance with results of **Perveen et al (1994)** and **Perveen and Kaiser (2007)**, **Tahavi (2000)** and **El-Naggar (2001)**. The usage of SEM in examination of seed coat of 11 taxa belonging to two species of *Hibiscus* revealed the importance of this technique as a good taxonomic tool. The results showed that a fairly heterogeneous seed coat patterns in the different species of *Hibiscus* and also



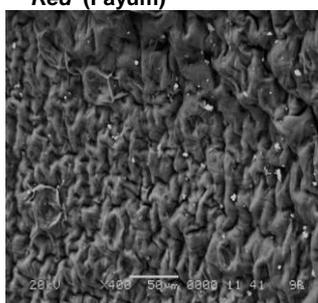
1- *H. esculentus* cultivar Red (Fayum)



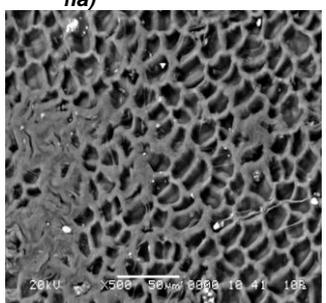
2- cultivar Municipal (Menoufia)



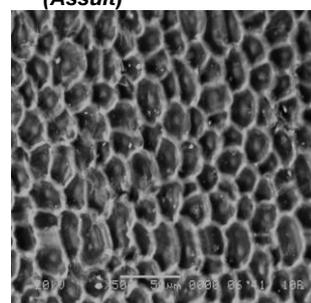
3- cultivar Musician (Assuit)



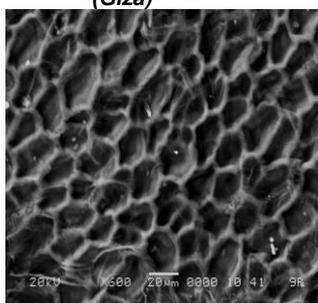
4- cultivar Municipal (Giza)



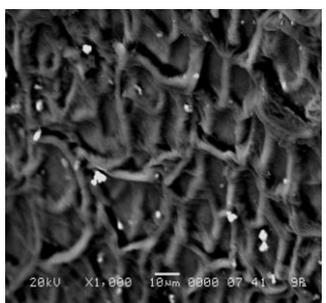
5- cultivar Red (Tanta)



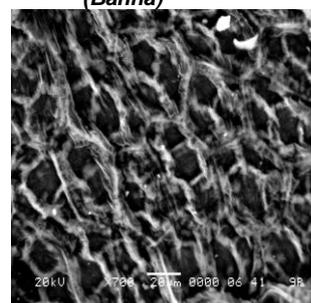
6- cultivar Ezabi without Bhuk (Banha)



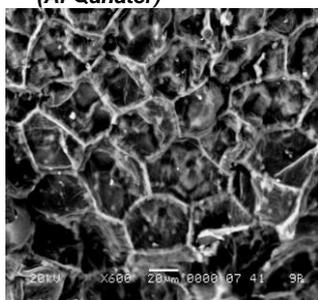
7- cultivar Ezabi Bhuk (Al-Qanater)



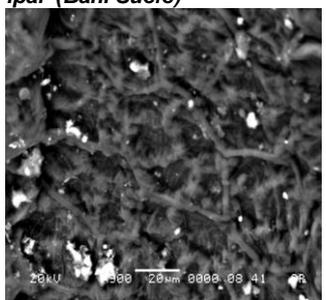
8- *H. sabdariffa* cultivar Municipal (Bani Suefe)



9- cultivar Ezabi (Tanta)



10- cultivar Municipal (Hosh Issa)



11- cultivar Ezabi (Hosh Issa)

Plate II. SEM photographs reveal seed morphological characters of the studied taxa of genus *Hibiscus* L.

Pollen grain and seed coat characters:

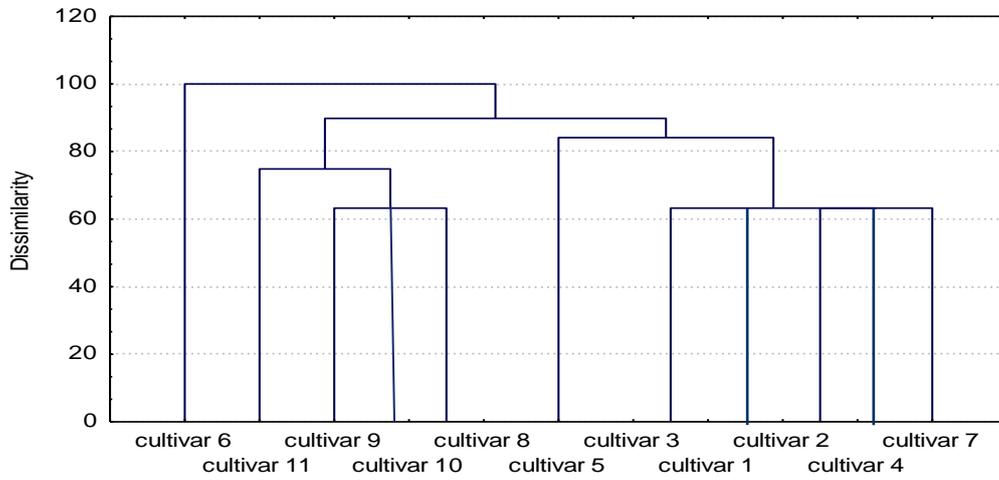


Fig. 1. UPGMA dendrogram based on SEM results of pollen grain morphology and seed exo-morphologic characters of the studied cultivars

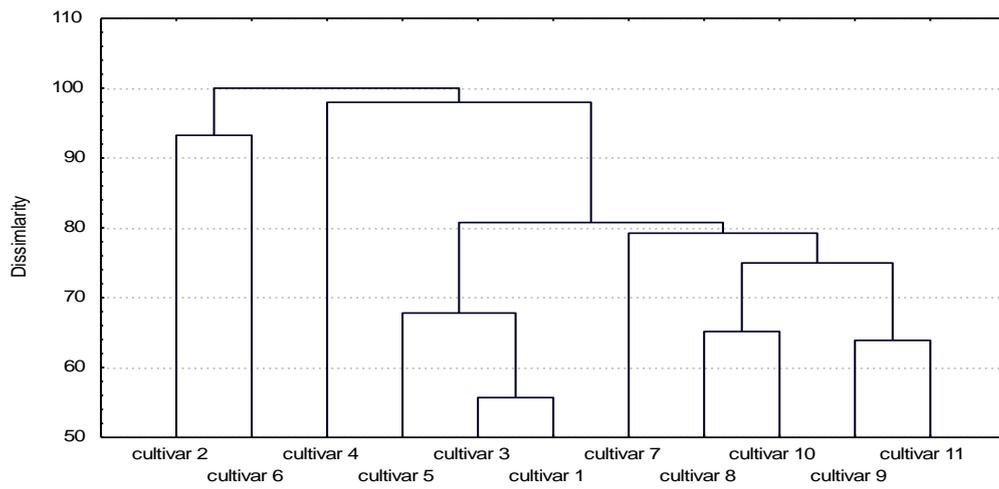


Fig. 2. UPGMA dendrogram based on seed protein profiles of the studied cultivars

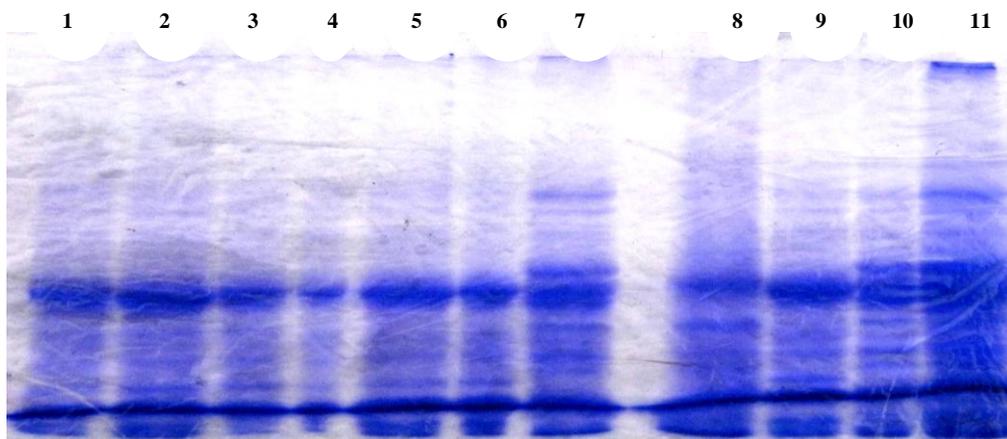


Plate III. SDS-Polyacrylamide Gel Electrophoresis illustrating Seed Protein bands of the Studied cultivars

Numerical Taxonomy:

Table 4. The resulted 37 binary characters of the studied taxa.(Characters & States are symbolized for numerical analysis)

Taxa	1	2	3	4	5	6	7	8	9	10	11
I- Pollen grain characters											
A- Pollen diameters											
1-Ranged from 60-70µm	0	0	0	0	0	0	0	1	1	0	0
2-Ranged from 71-80µm	0	0	0	0	0	0	0	0	0	1	1
3-More than 81 µm	1	1	1	1	1	1	1	0	0	0	0
B- Pore diameters											
4-Ranged from 4.5 to 5.0µm	1	1	1	1	1	1	1	0	0	0	0
5-More than 5.0µm	0	0	0	0	0	0	0	1	1	1	1
C- Pollen Shape											
6-Spherical	1	1	1	1	0	1	0	0	0	0	0
7-Not spherical	1	1	1	1	0	1	0	0	0	0	0
8-Pantaporate	1	1	1	1	1	1	1	1	1	1	1
D- Spinate Height											
9-Ranged from 10-11µm	0	0	0	0	0	0	0	1	1	1	1
10-More than 11 µm	1	1	1	1	1	1	1	0	0	0	0
E- Exine Thickness											
11-Ranged from 3.4-3.6µm	0	1	0	0	0	0	0	1	1	1	1
12-More than 3.6 µm	1	0	1	1	1	1	1	0	0	0	0
II- Seed Coat Characters											
A- Seed shape											
13-Depressed ovate	0	0	1	0	1	0	0	0	0	0	0
14-Round	0	1	0	0	0	1	0	0	0	0	0
15- Ovate	1	0	0	1	0	0	1	0	0	0	0
16-Kidney	0	0	0	0	0	0	0	1	1	1	1
B- Seed											
17-Black	1	1	1	1	1	1	1	1	0	1	1
18-Red	0	0	0	0	0	0	0	1	1	0	1
19-Pale red	0	0	0	0	0	0	0	0	1	0	0
C- Seeds Surface											
20-Smooth	1	1	1	1	1	1	1	1	0	1	1
21-rough	0	0	0	0	0	0	0	1	1	0	1
D- Seed size											
22-Less than 5.00 mm	1	0	0	0	0	0	0	0	0	0	0
23-More than 5.00 mm	0	1	1	1	1	1	1	1	1	1	1
E- Sculpture											
24-Regulate	1	1	1	1	1	1	1	0	1	0	0
25-Reticulate	1	1	0	1	1	1	1	0	1	1	1
26-Particles on the seeds	0	0	0	0	0	0	0	0	0	0	1
27-Oceolate	0	0	1	0	0	0	0	0	0	0	0
28-Foveolate	0	0	0	1	0	0	0	0	0	0	0
29-Ruminate	0	0	0	0	0	0	0	1	0	0	0
30-foveolate	0	0	0	0	0	0	1	0	0	0	0
31-Wax-like materials	0	0	0	0	0	0	0	0	0	1	0
III- Seed Protein Characters											
32- Rf ranged from 0.35 to 0.40	1	1	1	1	1	1	1	0	1	1	1
33- Rf ranged from 0.41 to 0.50	1	0	1	1	1	0	1	1	1	1	1
34- Rf ranged from 0.51 to 0.60	1	1	1	1	1	0	0	1	1	1	1
35- Rf ranged from 0.61 to 0.70	0	0	0	0	0	0	0	1	0	0	0
36- Rf ranged from 0.71 to 0.80	1	1	1	1	1	1	1	1	1	1	1
37- Rf ranged from 0.81 to 0.93	1	1	1	1	1	1	1	1	1	1	1

offer significant information to be used in infra-generic classification (Table 4). On the other hand, seed morphology showed variations to some extent in seed size, shape, and colour where colour varied from black in seven cultivars of *Hibiscus esculentus*, and pale red to dark red in the four cultivars of *H. sabdariffa*. The basic spermoderm pattern of the mature seed under SEM is regulated in some species but they show some variation in characteristics in each taxon. From our results, seed surface patterns with the 7 cultivars of *H. esculentus* fairly differ than those of 4 cultivars of *H. sabdariffa*. In addition, four types of seed sculptures were revealed from the SEM; reticulate (four cultivars of *H. sabdariffa* and four cultivars of *H. esculentus*), Ocellate recorded in *H. esculentus* cultivar Musician (Assuit), foveolate was recorded in *H. esculentus* cultivar Municipal (Giza) and Ruminant which recorded in *H. esculentus* cultivar Ezabi with Bhuk (Al-Qanater). Also, the study has established that the seed coat trichome structure is taxonomically significant. Each species has distinctive trichomes on the pollen grain. The spinate structure elucidated here for *H. sabdariffa* differs from which reported by Khushk and Vaughan (1986).

The electrophenogram produced from the analysis of the gel for the 11 cultivars revealed that the migration distance (RF) of the detected bands ranged from 0.35 to 0.94, the lowest migration distance of 0.35 (band No. 1) was detected in three cultivars of *H. esculentus* (Fayum, Giza & Al-Qanater) and three cultivars of *H. sabdariffa* (Bani Suefe, Tanta and Housh Issa). The highest migration distance of 0.94 (band number 31) was scored in the four cultivars of *H. sabdariffa*. It is clear that some bands are represented in a large number of species, while others are in a few accessions. A total number of 31 bands, a maximum number of 14 bands were estimated in *H. sabdariffa* cultivar Ezabi (Hosh Issa), 12 bands was estimated in *H. sabdariffa* cultivar Ezabi (Tanta), whereas the lowest band number was recorded in *H. esculentus* cultivar Ezabi without Bhuk (Banha) and *H. esculentus* cultivar Ezabi with Bhuk (Al-Qanater). The band number 19 and 30 were found in all the studied taxa of genus *Hibiscus*.

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