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ORIGINAL ARTICLE

Taxonomic evaluation using pollen grain sculpture and seed coat characters of 11 taxa of genus *Hibiscus* (Malvaceae) in Egypt

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Abstract Pollen grain morphology and seed coat characters of 11 cultivars belonging to two species of genus *Hibiscus* (Family Malvaceae) namely *H. esculentus*, *H. abelmoschus* 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, ocealate, foveolate and ruminate. Sodium Dodecyl Sulfate 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 that 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.

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 region. Characters illustrated from seeds as a protein and as an isozyme have been utilized in plant taxonomy at different hierarchical levels to construct the phonetic classification (Abd El-Hady, 2007). Christensen (1986) stated that the generic delimitation based on pollen morphology is difficult in this genus. El-Naggar (2004) studied the pollen morphological characters of 21 species of Egyptian flora.

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 and Qaiser (2007) reported that the present pollen data are

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

Studied species	Cultivar	Location	Section
H. esculentus L.	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
H. sabdariffa L.	Municipal	Bani Suefe	Furcaria
	Ezabi	Tanta	Furcaria
	Municipal	Hosh Issa	Furcaria
	Ezabi	Hosh Issa	Furcaria

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). Polyacralyamide Gel Electrophoresis for protein and isozyme was used for identification of species, subspecies and variety level (Adrianse et al., 1969; 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 accessions belonging to two species of genus *Hibiscus* L. were investigated and the sources of these taxa are given in Table 1.

SEM techniques

Pollen grains were collected after cultivation in Egypt to obtain pollen grains of buds and stored in the 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 30 nm layer of gold using fine coat ion sputter JEOL-JFC-1100E ion-sputtering device at an 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 h 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 an accelerated voltage of 15 kV at the Scanning Electron Microscopy Unit in Al-Azhar University.

Electrophoretic techniques

Extraction and analysis of seed protein fractions were carried out by using one dimensional Sodium Dodecyl Sulfate 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 fair 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 *Hibiscus 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 and 85.5 μm were recorded in cultivar *Musician* (Assuit) and cultivar Ezabi Bhuk (Al-Qanater), respectively. In addition the values of 84.50 and 82.5 μm were recorded in cultivar *Municipal* (Menoufia) and cultivar Red (Tanta) (Plate I(1–7)). On the other hand, the pollen grain diameter in *Hibiscus sabdariffa* cultivars ranged from 63.5 to 76.5 μm, where the highest value of

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

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