

**MORPHOLOGICAL STUDIES ON LEAF, POLLEN GRAIN AND SEED
OF GENUS *Oenothera* L. IN EGYPT BY USING
SCANNING ELECTRON MICROSCOPE "SEM"**

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**By
H. S. Abd – El Maksoud**

*Flora and Phyto-taxonomy Researches Department, Horticultural Research Institute,
Agricultural Research Center, Giza, Egypt..*

ABSTRACT

This study was performed on three species of the genus *Oenothera* in Egypt. These species are; *O. biennis*, *O. drummondii* and *O. speciosa*. The aim of this study was to distinguish the similarity and dissimilarity between these species. This study includes a number of taxonomic evidences and characters to establish the relationships among the above mentioned species. These characters were; 1) the morphological descriptions of the whole plants representing these species, 2) morphological descriptions and Scanning Electron Microscope (SEM) survey on the leaf, pollen grain and seed surfaces of the studied species. .Then proposed keys were designed.

Key words: *botanical key, leaf morphology, Oenothera, pollen grain , morphology ,seed morphology, SEM, taxonomy.*

1. INTRODUCTION

Oenothera is a genus of about 125 species of annual, biennial and perennial herbaceous flowering plants, native to North and South America. It is the type genus of the family Onagraceae, the Evening Primrose Family. (Quattrocchi, 2000). Leaf alternate, flower actinomorphic, axillary, solitary, sometimes forming a leafy terminal spike. Sepals 4, often caducous, usually strongly reflexed. Petals 4, yellow, rarely white or pink, abovate or obcordate, with or without a short claw. Stamens 8, equal or the inner ones shorter. Ovary 4-celled, cylindrical; stigma deeply 4-cleft into linear lobes or rarely capitate. Capsule splitting loculicidally into 4 valves. Seed without a tuft of hairs. (Zohary, 1987).

The size and shape of juvenile leaves in particular are important taxonomic characters in *Eucalyptus* (Pryor 1976) with closely related species being differentiated by these traits (e.g., Phillips and Reid 1980 and Potts and Reid 1985) . Ostroumove (1990) studied the stomata types on leaves of some species belonging to the tribes Coriandreae and Scandiceae (Umbelliferae)

in relation to taxonomy. Szujko-Lacza (1994) studied the leaf characters of *Coriandrum sativum*. El-Khanagry (2003) purposed a key to identify 49 species of grasses belonging to 33 genera by using vegetative characters of leaves as well as trichomes and cuticular ornamentations. However, the gross morphological characters of the leaf have been used for identification purposes. With increased sophistication of classification systems it has become increasingly important to have more elaborative means for identification. The leaf has not lost its importance as a taxonomic tool but rather has proved to be more useful when a full understanding of all its characteristics are known and appreciated.

Pollen grain shapes are described, as well as markings, size, color, stickiness, abundance and other features. It is found that, in general, the shape and size of grains in the various genera exceptions are the thread-shaped pollen grains of some eelgrass) and in aspect: round, ovale, disc or bean-shaped and sometimes filamentous. The natural color is mostly white, cream, yellow or orange. The texture of the cell

wall shows also great variations, from smooth to spiky (Maxy, 1925). Defining sculpture of those forms have been described only the basis of observations using TEM and SEM (Basil, 1995). Pollen grains of various species can vary quite a lot in size (from about 10 to nearly 100 micrometer) and shape; round, ovate, disc or bean-shaped and sometimes filamentous. The natural color is mostly white, cream, yellow or orange. The texture of the cell wall of pollen grain shows also great variations, from smooth to spiky (Thanikaimoni and Van derHam, 1999).

Fossil pollen grains are distinguished primarily by their form and their surface sculpture furnishing a Key to distinguish 17 basic pollen classes (Christopher 1979).

Pollen of 110 species from 18 genera in the Portulacaceae has been examined by light and scanning electron microscopy, and a representative number by transmission electron microscopy. (Bio 1992). Pollen grains of the neotropical subtribe Cuspariinae (Rutaceae) were examined by LM, SEM and TEM. The pollen morphology of this subtribe is very diverse. (Cynthia and Jacquelyn, 1993).

The great variations in the morphology and the different ornamentations of the seed coat support the study of taxa delimitation and may solve and facilitate many taxonomic problems. Netolizky (1926) confirmed also that the morphology of seed coat surface should be the principles of natural classification of the flowering plants. Vaughan (1968) suggested that the structure of the mature seeds, especially the coat structure is considered the more taxonomic useful information.

Surface sculpturing may aid in solving problems of identity or relationship concerning taxa at various levels (Werker, 1997). The SEM examinations of seed surface features could be applied in taxonomy and there are many

characters (e.g. seed coat) that could be used to characterize groups of related species, genera or taxonomic categories up to the sub-family levels. Some characters of the micro-morphology and orientation of epicuticular wax crystalloid are surprisingly of high systematic significance (Barthlott, 1981). More detailed structural information about seeds are obtained by using the Scanning Electron Microscope. However, Yeh and Kakuma (1990) suggested that seed characters; colour, coat patterns, shape, size, outer arils and hilum lead to better criteria for species identification and support the taxonomical positions of taxa.

The aim of this study was to distinguish the taxonomic relationship among three species of genus *Oenothera* in Egypt namely *O. biennis*, *O. drummondii* and *O. speciosa*.

2. MATERIALS AND METHODS

Three species of the genus *Oenothera* were studied (Table 1). The fresh leaves, pollen grain and seeds of each species were used. These species were planted in the Medicinal and Aromatic Plants Research Department, Horticultural Research Institute, Agricultural Research Center, Dokki, Giza. The detailed surface scan features were examined by using Scanning Electron Microscope (SEM) with different magnifications. Scanning was carried by JEOL- JSM T 100 Model Scanning Electron Microscope, Central Laboratory, National Information and Documentation Center (NIDoC), Dokki, Giza, Egypt.

3. RESULTS AND DISCUSSION

The results are gathered according to the following aspects: macromorphological and Scanning Electron Microscope (SEM) descriptions of leaf (upper and lower surfaces), pollen grain and seed surfaces for each species.

Keys were proposed to distinguish

Table (1): The studied species and the collection regions.

No.	Species	Habit	Region
1.	<i>Oenothera biennis</i> L.	Cultivated	Experimental field of Faculty of Agriculture, Cairo University and Agricultural Museum, Giza.
2.	<i>Oenothera drummondii</i> Hook. (= <i>O. humifusa</i> Nutt.)	Wild	North Manzala Lake- near Boughaz El Gameel.
3.	<i>Oenothera speciosa</i> Nutt.	Cultivated	Orman garden and Agricultural Museum, Giza.

the studied species based on morphological characters of the whole plants and macro and micromorphological characters of the leaf and seed of the studied species.

1) *Oenothera biennis* L.

Biennial, stem erect growing to 30–200 cm tall, ramified only above, dense leafy, hairy, glandularly downy. Inflorescence erect. Flowers large, yellow, 2.5–5 cm in diameter, grow all along the stalks. Sepals – apices terminal, slender, appressed to one another in bud. Petals four, hermaphrodite. The fruit is a capsule 2–3.2 cm long and 4–6 mm broad, ovate, tapering above, numerous seeds, released when the capsule splits into four sections at maturity.

i) Leaf

Lanceolate or oblong- lanceolate, 5–19 cm long and 1–5 cm broad, produced in a tight rosette in the first year, and spirally on the stem in the second year, green colour, covered with short hairs on both surfaces. Upper epidermis has stomatal type; anomocytic with semidepressed level and with scrobiculate sculpture pattern. Lower epidermis has anomocytic stomatal type with superficial and semiraised level and with ruminant sculpture pattern. Trichomes; non-glandular (vinca) and glandular (vasaka), tuberculate ornamentation.(Fig. 1 and 2).

ii) Pollen grain

Pollen grain free, more than one aperture, tricolporate, normapollis - type; plicapollis. The zone of germination pores depressed. Tectum glebulate.(Fig. 4).

iii) Seed

Seed numerous 1–4 x 0.7-1 mm in dimensions , released when the capsule splits into four sections at maturity. Variously angled, often with a rounded side, or angular, spindle shaped to flattish-oblong, or angular – lanceolate; smooth to rough surfaced, yellow – brown to dark purplish brown, surface dull, glabrous, fine waved. Colliculate epidermal cell walls. Depressed anticlinal walls and usually 5-6 gonial, raised boundaries, straight with irregular canals. Outer periclinal walls are convex or concave with delicate furrows. Hilum inconspicuous, basal, rough and hairy. (Fig. 3).

2) *Oenothera drummondii* Hook. (= *O. humifusa* Nutt.)

Perennial, pubescent herb, shrubby, up to 80 cm tall. Stem many, erect or

procumbent, much branched all along. Flower 5- 7 cm, across. Sepals 2-3 cm, reflexed, linear- lanceolate. Petals 2.5 – 3 cm ,bright yellow, broadly obovate. Stigma deeply 4 cleft. Capsule 2.5 – 5 cm long and 2 – 4mm broad , lanceolate, obtuse 4 angled.

i) Leaf

Lanceolate – oblong, 3–6 x 1-2 cm, tapering gradually or abruptly to a short petiole, acute or obtuse, entire or slightly toothed, densely soft- pubescent covered with short white hairs, silvery green. Upper epidermis has stomatal type; anomocytic with superficial level. Ruminant sculpture pattern of upper epidermis. Lower epidermis has anomocytic stomatal type with depressed level. alveolate sculpture pattern. Trichomes; non-glandular (datura) and glandular (vasaka), smooth ornamentation.(Fig. 1).

ii) Pollen grain

Pollen grain free, more than one aperture, tricolporate, normapollis - type; semioculopollis. The zone of germination pores raised. Tectum glebulate-favulariate. (Fig. 4).

iii) Seed

Numerous fusiform seed / capsule. 1–1.4 x 0.4-0.7mm in dimensions , often with a rounded side, oblong shaped, smooth surfaced, cream to brown, surface glossy, glabrous. Reticulate epidermal cell walls. Raised anticlinal walls and usually 4-5 gonial, slightly raised boundaries and sinuous with irregular canals. Outer periclinal walls discharged. Hilum conspicuous, basal, glabrous, smooth. (Fig. 3).

3) *Oenothera speciosa* Nutt.

Herbaceous, perennial, wildflower, glabrous (smooth) to pubescent stems that grow to 40 cm in height. Flower single, four-petaled, cup-shaped flowers on the upper leaf axils, fragrant shell-pink flowers, 1.5–2 x 3 –5 cm flowers start out white and grow pink as they age. The flower throats, as well as the stigmas and stamens, have a soft yellow color. Capsule 0.5 – 1.1 cm long and 2 – 3mm broad , lanceolate, carpophore present.

i) Leaf

Pubescent, alternate , sessile or petiolate , reaching 1 x 4cm. Variable in shape, from linear to obovate, and are toothed or wavy-margin, green, base of blade distanced.

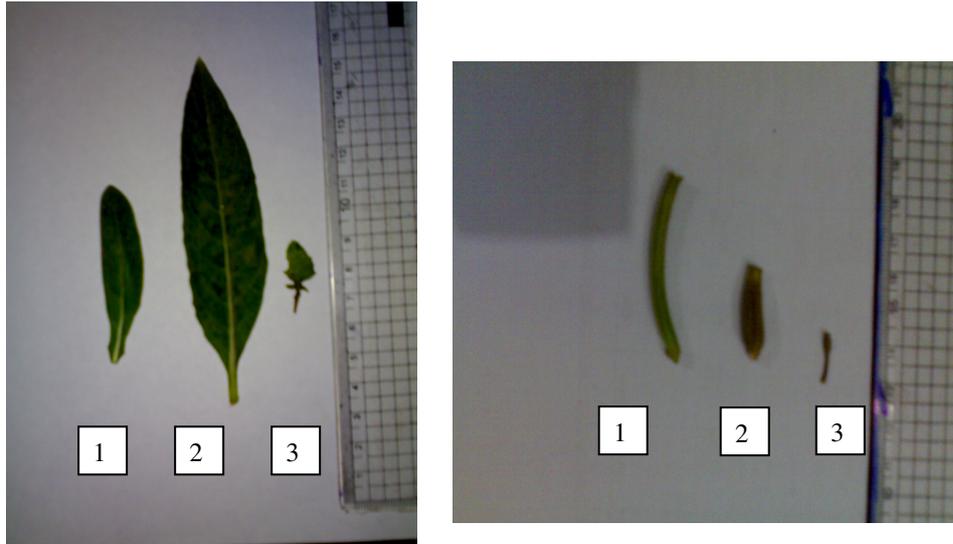


Fig.(1): Shapes of leaf (left) and capsule (right) of the studied species.
1) *Oenothera drummondii* 2) *O. biennis* 3) *O. speciosa*

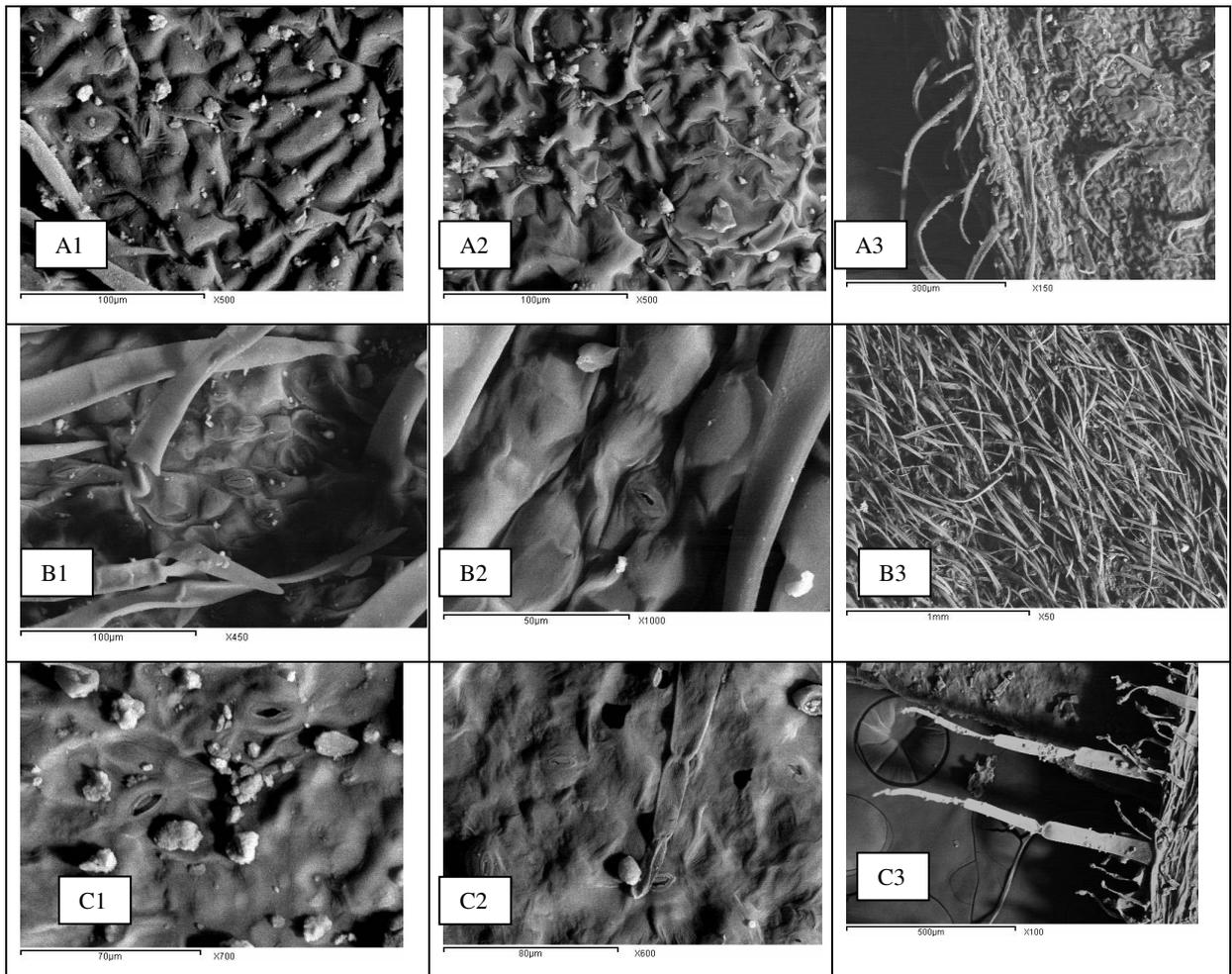


Fig. (2): SEM on leaf epidermis; (1)upper surface,(2) lower surface (3) trichomes
A) *Oenothera biennis* B) *O. drummondii* C) *O. speciosa*

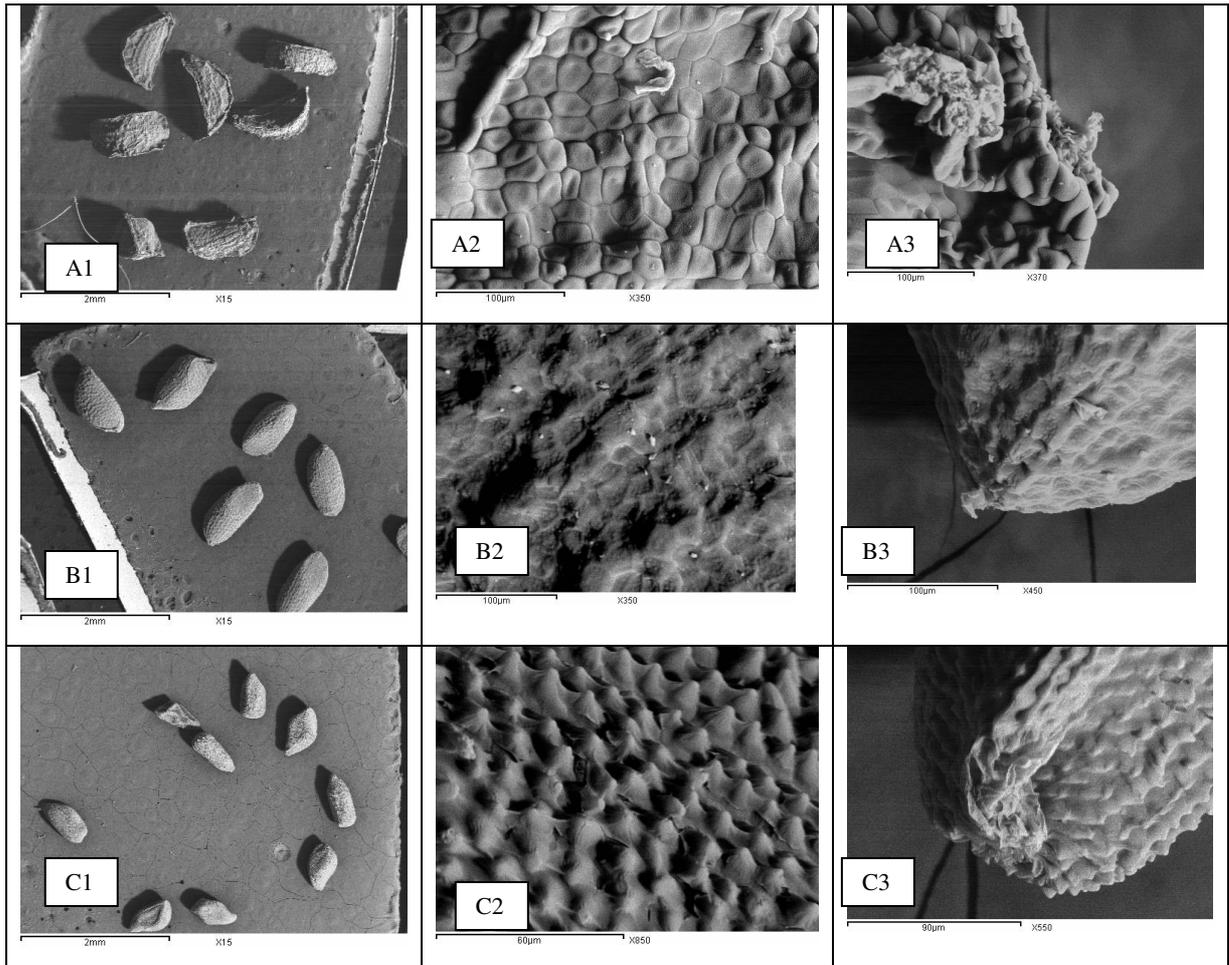


Fig. (3): SEM on seed; shape(1), surface sculpture(2) and hilum(3) of the studied species. A) *Oenothera biennis* B) *O. drummondii* C) *O. speciosa*

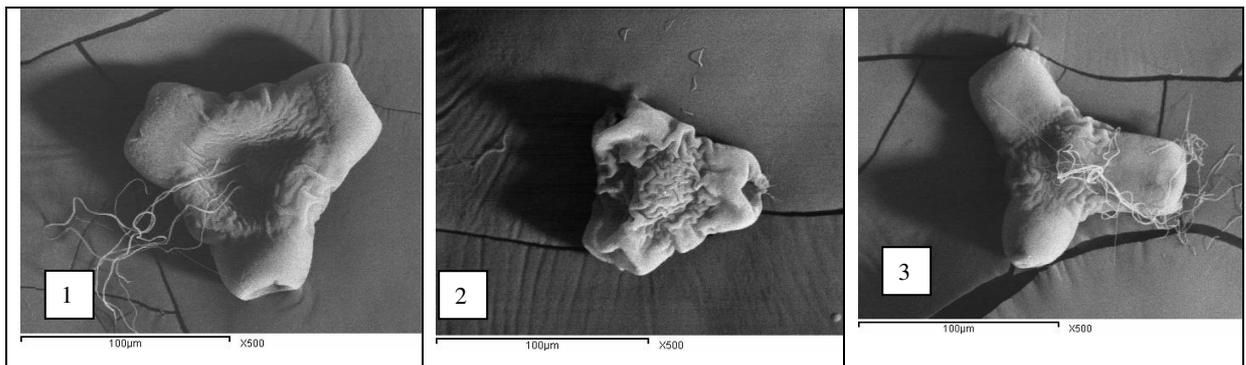


Fig. (4): SEM on pollen grain of the studied species. 1) *Oenothera biennis* 2) *O. drummondii* 3) *O. speciosa*

Upper epidermis has stomatal type; anomocytic with superficial level. Pusticulate sculpture pattern. Lower epidermis has anomocytic stomatal type with depressed level. Pusticulate sculpture pattern. Trichomes; non-glandular (*datura*) and glandular (*belladonna*), smooth ornamentation. (Figs. 1 and 2).

ii) Pollen grain

Pollen grain free, more than one aperture, tricolporate, type tricolpate. The zone of germination pores semiraised. Tectum glebulate. (Fig. 4).

iii) Seed

Seed numerous 0.6–1 x 0.3- 0.5 mm in dimensions, often with a rounded side, ovate

shaped, smooth surfaced, cream, surface shiny, glabrous. Tuberculate epidermal cell walls. The anticlinal walls elongate in one direction and usually 4 gonal, boundaries raised, straight. Outer periclinal walls convex. Hilum conspicuous, basal, glabrous, smooth. (Fig. 3).

Keys

The following keys were proposed to distinguish and identify the studied species. The skeleton of these keys are based on; 1) the general morphological features of the whole plant of studied species and 2) the macro and micro morphological characters of the seed and leaf.

1) Key based on the morphological characters of the studied species.

A. Flower yellow

b. Capsule 2–3.2 cm x 4–6 mm, ovate.

Biennial, herbaceous..... *Oenothera biennis*

bb. Capsule 2.5–5 cm x 2–4 mm, lanceolate.

Perennial, shrubby. ... *O. drummondii*

AA. Flower pink, herbaceous perennial. The flower throats, as well as the stigmas and stamens, have a soft yellow. Capsule 0.5–1.1 cm x 2–3 mm, lanceolate. Carpophore present..... *O. speciosa*

2) Key based on the macro and micro morphological characters of the leaf of the studied species.

A. Leaf small, sessile or petiole, reaching 1 x 4 cm, toothed or wavy-margin, green, base of blade distanced. Pusticulate sculpture pattern of upper and lower epidermis..... *O. speciosa*

AA. Leaf not so.

a. Surface of leaf silvery green, 3–6 x 1–2 cm, lanceolate – oblong. Ruminant sculpture pattern of upper epidermis with superficial level of stomata. Lower epidermis alveolate with depressed level of stomata..... *O. drummondii*

aa. Surface of leaf green, 5–19 x 1–5 cm, lanceolate or oblong-lanceolate. Scrobiculate sculpture pattern of upper epidermis with semidepressed level of stomata. Lower epidermis ruminant with superficial and semiraised stomatal level..... *O. biennis*

3) Key based on the macro and micro morphological of seed of studied species.

A. Seed rounded side

a. Cream to brown, oblong shaped. Reticulate epidermal cell walls. Outer periclinal walls discharged..... *O. drummondii*

aa. Cream, ovate shaped. Tuberculate epidermal

cell walls. Outer periclinal walls are convex..... *O. speciosa*

AA. Seed variously angled, often with a rounded side, or angular, spindle shaped to flattish-oblong, or angular – lanceolate, yellow – brown to dark purplish brown, surface fine waved. Colliculate epidermal cell walls. Outer periclinal walls are convex or concave with delicate furrows..... *O. biennis*

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دراسات مورفولوجية على الورقة وحبوب اللقاح والبذرة لجنس *Oenothera* L في مصر
باستخدام المجهر الماسح الإلكتروني

حنان سلامة عبدالمقصود

قسم بحوث الفلورة وتصنيف النباتات - معهد بحوث البساتين - مركز البحوث الزراعية - مصر

ملخص

يضم جنس الأونثرا حوالي ١٢٥ نوعا في العالم ويوجد بمصر ثلاثة منها نوعا برياً وهو *Oenothera drummondii* واثنان منزرعان هما: *O. speciosa* و *O. biennis* وقد أمكن استخدام الصفات المورفولوجية وصفات الورقة والبذرة وحبوب اللقاح باستخدام المجهر الإلكتروني الماسح لتمييز هذه الأنواع ومحاولة عمل توثيق وحصر معلوماتي لأنواع الأونثرا الموجودة في مصر بهدف استخدامها كمعايير ودلائل تقسيمية للتعرف على هذه الأنواع مستقبلاً.

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