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Pollen diversity among the inconstant male *Ochradenusbaccatus* delile populations in Egypt

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Abstract

In Egypt, Resedaceae is represented by 14 species belonging to five genera, genus *Ochradenus* is among them represented by one species (*O. baccatus* Delile). The studied *Ochradenus baccatus* populations collected from Wadi Degla, Egypt revealed that *O. baccatus* is gynodioecious species, with female individuals that are constant in sex expression and males that exhibit great variation in functional gender. The field study revealed the presence of inconstant male of *O. baccatus* in different forms ranging from high fruit-producing to low or non-fruit producing forms with two intermediate forms. The pollen morphology of all the four forms was examined using LM and SEM. This study is the first dealing with pollen grain morphological diversity within different *O. baccatus* populations, representing model of the inconstant male of a gynodioecious species. Generally, the examined *Ochradenus baccatus* pollen grains appeared under SEM as 3-colporate, isopolar, radially-symmetrical, with reticulate exine sculpturing. This study revealed the presence of a variability in pollen size, shape, aperture and exine sculpturing in different forms. The polymorphic pattern observed in inconstant *O. baccatus* male populations in Egypt was attributed to internal factors, the polyploidy phenomenon was among them and the external factors were excluded.

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Introduction

Palynology is an interdisciplinary science which has a tremendous contribution to the systematics and phylogeny of angiosperms (Perveen, 2000). The constant features and sculpturing of the exine make pollen grains a highly recognizable object (Moore and Webb, 1978). The most important pollen phylogenetic information involve the number, position, structure of germination apertures, exine structure and stratification (Walker and Doyle, 1975).

The palynology of the family Resedaceae is quite heterogenous (Perveen and Qaiser, 2001), pollen of this family has been examined earlier by several authors among them: Allen (1937), Erdtman (1952), Kuprianova and Alyoshina (1972), Hodges (1974), Rao and Leong (1974), Arbo (1974), Moore and Webb (1978), Mitra and Mitra (1979), Valdes *et al.* (1987), Moore *et al.* (1991) and Punt and Marks (1995). The pollen morphology of the family Resedaceae is significantly useful at the generic level, Perveen and Qaiser (2001) examined the pollen morphology of four Resedaceae species belonging to 3 genera; they recognized 2-distinct pollen types, namely I: Tricolpate-type (in genus *Ochradenus*) and II: Tricolporate-type (in *Oligomeris* and *Reseda*). In Egypt, family Resedaceae is represented by 14 species belonging to five genera, among them genus *Ochradenus,* which represented by one species namely *O. baccatus* Delile (Täckholm, 1974; El Hadidi and Fayed, 1994/95 and Boulos, 1999).

No earlier studies was carried out to elucidate the pollen features of the inconstant male forms of *Ochradenus baccatus*. Accordingly, this study is a pioneer study aimed to clarify the diversity in: pollen size, shape, aperture and exine sculpturing in different forms of the inconstant male using SEM, to deduce the possible role of pollen diversity in male evolution in this gynodioecious species.

Materials and methods

Plant material

Representative flowering plants of the studied *Ochradenus baccatus* Delile population forms were collected from Wadi Degla (29° 56′ N, 31° 29′ E), in the northern part of the eastern desert of Egypt during March 2014.

The representative male (inconstant male) specimens of *Ochradenus baccatus* were sorted morphologically into four forms (F1- F4), based on the degree of inconstancy in male inflorescence ranging from high fruit producing form (F1) to very low or non-fruit producing form (F4). More detailed description will be outlined in the result section.

Fresh anthers were collected from the floral buds of studied *Ochradenus baccatus* population forms. Anthers were fixed in FAA (50 ml ethyl alcohol, 10 ml formaldehyde, 5 ml glacial acetic acid, 35 ml distilled water) for examination.

Samples preparation for Scanning Electron Microscope (SEM)

The anthers under investigation were removed from FAA and then placed through an ETOH dehydration series, dried with a Denton DCP-1 apparatus, mounted and coated with 600 Å of Gold-Paladium mixture with a Technics Hummer V-Sputter Coater. Samples were scanned on a Jeol 1200 EX II SEM at 20 kv. Size measurements were obtained from the average of 10 randomly selected grains when possible. Lumina and muri diameter were measured and the ratio of the Polar axis to the Equatorial axis (P/E) was calculated. The used pollen terminology in this study followed Erdtman (1952) and Walker and Doyle (1975).

Results

Variability of the inconstant male inflorescence

The studied *Ochradenus baccatus* populations with inconstant male specimens were examined morphologically (Fig. 1), the results indicated that there was no notable variations in plant size, leaf and flower morphology except some of the inflorescence features. The number of flowers, fruits and seeds / inflorescence were counted including the flower scars.. The data as mean value were outlined in Table 1, the recognized forms were:

a. Form 1 (F1) The inflorescence was characterized by its dense raceme (Fig. 1), flowers number ranging from 42-62 / inflorescence, nearly half of this number (40%) were fruit-producing flowers (Table 1).

b. Form 2 (F2) The inflorescence in this form was less dense, flowers number was 25-45 / inflorescence, many flowers were falling down before fruit formation (Fig. 1), nearly 29 % of the flowers were fruit-producing (Table 1).

Table 1. Fruit production within the inconstant male forms of *Ochradenus baccatus*.

Male Forms	No. of flowers / inflorescence	No. of fruits / inflorescence	No. of seeds / inflorescence	% productivity
Male F1	52	21	126	% 40
Male F2	35	10	63	% 29
Male F3	42	0.25	1.5	% 0.006
Male F4	46	0.15	0.9	% 0.003

c. Form 3 (F3) The inflorescence in this form consisted mostly of deciduous flowers except few flowers at the top (Fig. 1). The number of flowers including flower scars was 32-52 / inflorescence. Limited number of fruits were present with a ratio not exceeding 1 % (Table 1).

d. Form 4 (F4) This population form appeared in field as functionally pure male (Fig. 1) with rare presence of fruit-producing flowers. The number of flowers was 36-56 acting totally as pure male with 2 or 3 fruits in the whole shrub (Table 1).

Table 2. Summary of pollen characters of the studied inconstant male forms of *Ochradenus baccatus* based on Scanning Electron Microscope (SEM).

Pollen character	Form 1	Form 2	Form 3	Form 4
P (µm)	15.56 -17.03 (16.29)	10.29 -16.67 (13.48)	12.96 -17.04 (15.00)	18.75 - 25.0 (21.89)
E (µm)	8.89 - 13.33 (11.11)	6.43 - 13.33 (9.88)	8.89 - 11.85 (10.37)	14.29 - 19.0 (16.65)
P/E	1.46	1.36	1.45	1.31
Colpus (L x w)	8-11.67 x 1.2-5.28 (9.83 x 3.24)	8.5-16.6 x 0.45-4.1 (12.62 x 2.31)	11.1-14.1 x 2.2-5.6 (12.65 x 3.93)	17.5-20.6 x 3.1-5.7 (19.06 x 4.44)
Lumina diam. (µm)	0.22 - 0.67	0.11 - 0.71	0.19 - 1.91	0.25 - 1.5
Muri width (µm)	0.19 - 0.37	0.21 - 0.64	0.22 - 0.63	0.38 - 0.71

P: Polar axis, E: Equatorial axis, L: Length, W: Width, the mean values given in parentheses.

Pollen features using LM

The examined *Ochradenus baccatus* Delile pollen grains using light Microscope (LM), revealed the presence of two shapes of pollen grains in polar view:

1. Circular (circulaperturate), which appeared in pollen grains of Form 1 (Fig. 2 a).

2. Slightly trilobed (fossaperturate), which appeared in pollen grains of Form 4 (Fig. 2 b), Form 2 and 3 were intermediates between these two forms.

Pollen features using SEM

Generally, the examined *Ochradenus baccatus* pollen grains appeared under SEM as 3-(colporate-colpate), isopolar, radially-symmetrical.The exine sculpturing was reticulate composed of irregular lumina and muri (Fig. 3).

a. Size

The pollen size of the studied *Ochradenus baccatus* inconstant male population forms were outlined in Table 2. The smallest pollen grains found in Form 2 with an average polar axis (PA) was 13.48 µm and average equatorial axis (EA) was 9.88 µm. While the largest PA with a value of 21.89 µm and EA of 16.65 µm were observed in Form 4 (Table 2).

b. Shape

Pollen grains of the studied *Ochradenus* forms were prolate , the short equatorial axisnot exceeding 16.65

µm and a longer polar axis up to 21.89 µm. The P/E ratio(Table 2) grouped the studied pollen shape into the following classes :

1. Subprolate (P/E ratio 1.15-1.33), this type was found in Form 4 (P/E = 1.31), as shown in Fig. 7.

2. Euprolate (P/E ratio 1.34-1.99), this type was found in all the remaining Forms (F1, F2 & F3), as shown in Figs. 4, 5 & 6.

Fig. 1. Inconstant male population forms according to fruit production.

F1: Form 1, F2: Form 2, F3: Form 3 and F4: Form 4

The investigated pollen grains appeared with three elongate, furrow-like, equatorial, meridional apertures. In Form 4, they appeared tricolpate, while, they appeared tricolporate with circular, lolongate ora in the remaining forms (F1, F2 & F3; Figs. 4, 5 & 6). . The colpus membrane appeared simple in Form 4 (Fig. 7), and compound, densely granulate in the remaining forms (Figs. 4, 5 & 6). Dimensions of colpi were variable in length and width among the investigated forms. Form 4 had the longest and widest colpus (with a mean length 19.06 µm and a mean width 4.44 µm). While, Form 1 possessed the shortest colpus (9.83 µm). . Colpus length of the remaining forms was medium-sized among the former measurements (Table 2).

d. Exine structure and sculpturing

The exine type of the investigated *Ochradenus baccatus* forms was semitectate, columellate, consisted of an open reticulum. The reticulate exine appeared heterobrochate due to the irregularity in lumina size and shape (varies from polygonal, to circular or indefinite shape). The largest lumina located near the equator and reduced gradually towards poles and colpi margins as shown in Figs. 4, 5, 6 & 7. According to the lumina diameter (Table 2), all the investigated forms had finely-reticulate exine with lumina diameter ranged from 1-2 µm. The intraluminal baculae were found in Form 1 (Fig. 4 c) and Form 4 (Fig. 7 c), and absent in Forms 2 and 3 (Fig. 5 c & Fig. 6 c). The muri appeared straight, thinner than the lumina with width < 1 µm in all the studied forms.

Discussion

Palynology of family Resedaceae is quite heterogenous (Perveen and Qaiser, 2001). The present study was dealing with the morphological variability of pollen grains of the inconstant *Ochradenus baccatus* male populations in Egyptian desert wadi using SEM. The results revealed the presence of four morphologically distinct inflorescence forms given the symbols: Form 1 to Form 4; as shown in Fig. 1. The degree of male inconstancy ranging from high fruit production (Form 1, Fig. 1) to very low or non-fruit production (Form 4, Fig. 1). The studied *Ochradenus baccatus* populations collected from Wadi Degla, Egypt revealed that *O. baccatus* is gynodioecious species, with female individuals that are constant in sex expression and males that exhibit great variation in functional gender. Fig. (1) outlined that males ranges from individuals that obtain all of their fitness via male characters as seen in Form 4 (seed productivity is 0.003 %; Table 1, Fig. 1) to that individuals which produces as many seeds as pollen as seen in Form 1 (with seed productivity 40 %; Table 1, Fig. 1). In addition to intermediate forms (Table 1, Fig. 1). All these forms named earlier by Wolf and Shmida (1997) as “inconstant males”, they observed the same phenomenon in *O. baccatus* populations from Israel. In this study, all the populations forms were collected from the same locality on the same date (see the materials section), accordingly the observed pollen variability in the studied populations will be related here to internal factors (genetic, or evolutionary mutation,.....) and not related to environmental factors or aridity gradient as explained earlier by Wolf and Shmida (1997) who related the functional male gender variability to environmental issues.

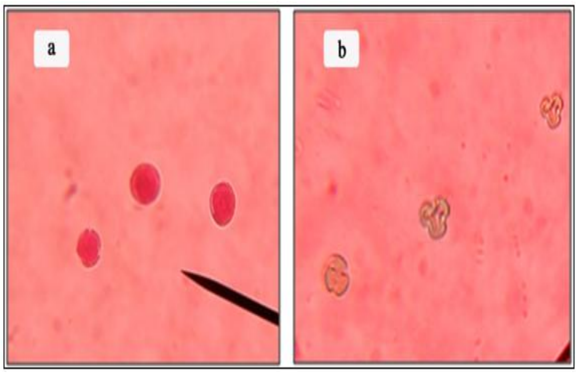

Fig. 2. Light micrographs showing *Ochradenus baccatus* pollen grains shape in polar view from: **(a):** Form 1 (highly fruit-producing form), **(b):** Form 4 (rarely fruit-producing form).

The light microscope study revealed the presence of circular or slightly trilobed pollen in high-fruit producing form (as in Form 1, Fig. 2), while the pollen of functionally pure male form (as in Form 4, Fig. 2) appeared fossaperturate or trilobed in polar view. These results indicated that the pollen grains of Form 1 were less fertile and less developed than that of Form 4. This postulation was supported by Spigler and Ashman (2011), who claimed that in gynodioecious species where males and hermaphrodites can be distinguished, males tend to have greater male fertility than hermaphrodites. The male fitness evolved from Form 1 to Form 4.

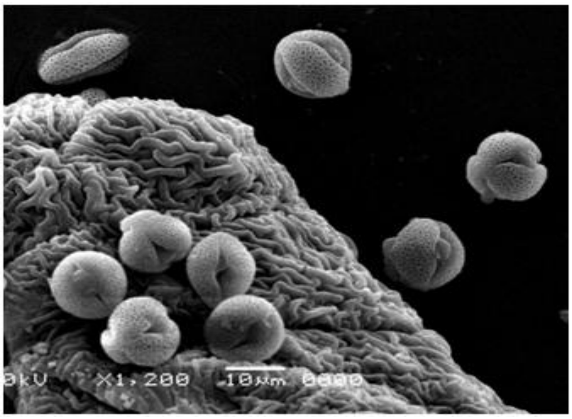

Fig. 3. *Ochradenus baccatus* pollen grains under SEM.

The pollen grain size of all the studied *O. baccatus* forms were included under the small-sized class (Table 2), according to Walker and Doyle (1975) classification, the largest size among them found in Form 4 (P= 21.89 μm; Table 2), while the smallest size found in Form 1 (P= 16.29; Table 2); these data supported our postulation of the increase in male fitness from Form 1 to Form 4 through Forms 2 and 3 as intermediates.

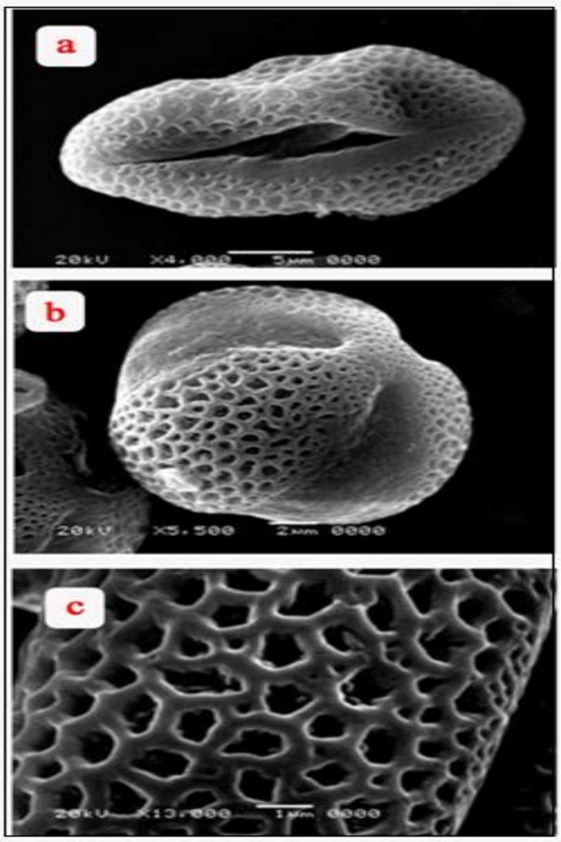

Fig. 4. SEM micrographs of *Ochradenus baccatus* inconstant male flower, Form 1 showing tricolporate pollen grains; **(a):** equatorial view, **(b):** oblique polar view, **(c):** detail of ornamentation.

The shape of the studied *O. baccatus* populations appeared to be in two groups: subprolate (P/E 1.15-1.31; Table 2) as in Form 4 and euprolate (P/E 1.34-1.99; see results section) in other forms (Forms 1, 2 & 3; Table 2). These results disagreed with the earlier studies carried out by: El Ghazaly (1991) who claimed that the pollen of *O. baccatus* had prolate spheroidal shape in his study on the flora of Qatar, also with El Naggar (2002) who treated it as perprolate in shape, his specimens were collected from Southern Egypt (Wadi Al Assiuty). This disagreement between the present study and El Naggar (2002) may be related to the presence of inconstant males in many forms related to the different populations in grown in Egypt. The aperture characters could distinguish between inconstant male Form 4 which appeared tricolpate with simple membrane structure (Fig. 7) from the other forms namely, Forms 1, 2 & 3; which appeared tricolporate with compound densely granular membrane (Figs. 4, 5 & 6). This result disagreed with El Naggar (2002) and El Ghazaly (1991) both considered *Ochradenus baccatus* pollen in general as tricolporate with compound granular membrane, while Perveen and Qaiser (2001) considered it in general as tricolpate. This finding contradicted with the evolution line which considered tricolpate as a basic form from which the tricolporate pollen has evolved.

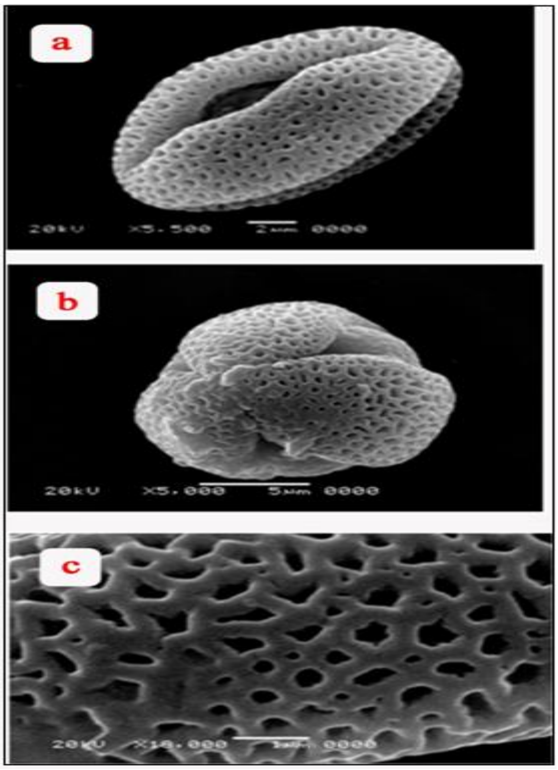

Fig. 5. SEM micrographs of *Ochradenus baccatus* inconstant male flower, Form 2 showing tricolporate pollen grains **(a):** equatorial view, **(b):** oblique polar view, **(c):** detail of ornamentation.

Exine sculpturing appeared finely-reticulate in all the studied forms (F1-F4, Figs. 4, 5, 6 & 7). This disagreed with Perveen and Qaiser (2001) and El Naggar (2002) which considered it as coarsely-reticulate. Also, the reticulum appeared heterobrochate with irregular lumina size. This results agreed with Perveen and Qaiser (2001) while disagreed with El Ghazaly (1991) who stated that the *O. baccatus* pollen grain has homobrochate reticulum. The presence of intraluminal baculae in Forms 1 & 4 (Figs. 4 c & 7 c) could differentiate them from Forms 2 & 3 (Figs. 5 c & 6 c). In the same time, the density of these baculae could differentiate between Form 1 (with few intraluminal baculae) and Form 4 with dense intraluminal baculae.

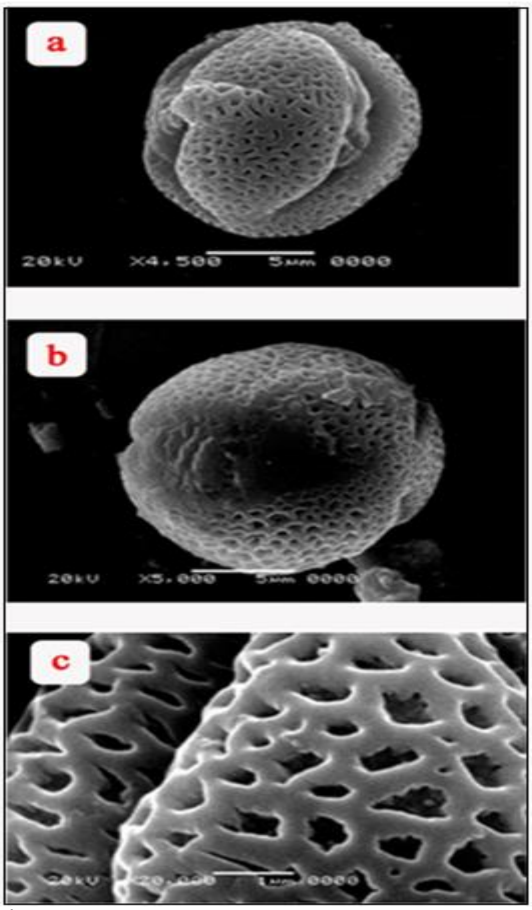

Fig. 6. SEM micrographs of *Ochradenus baccatus* inconstant male flower, Form 3 showing tricolporate pollen grains **(a):** equatorial view, **(b):** oblique polar view, **(c):** detail of ornamentation.

The pollen morphological diversity within *O. baccatus* (gynodioecious species) as far as the author knows has not been subjected to earlier detailed SEM study at the forms level. This study revealed the presence of a variability in pollen size, shape, aperture and exine sculpturing. These results are supported by the earlier work carried out by Knapp *et al.* (1998) on twelve of the thirteen known dioecious species of

Solanum. He observed that the pollen morphology of the functionally male flowers was more or less uniform, with the typical tricolporate pollen found in most species of *Solanum,* while the pollen of the functionally female flowers was typically inaperturate with a thicker exine and a wide range of surface ornamentation. He suggested that a number of different modifications to the basic developmental pathways of pollen formation were involved and concluded that dioecy has arisen several times within the genus.

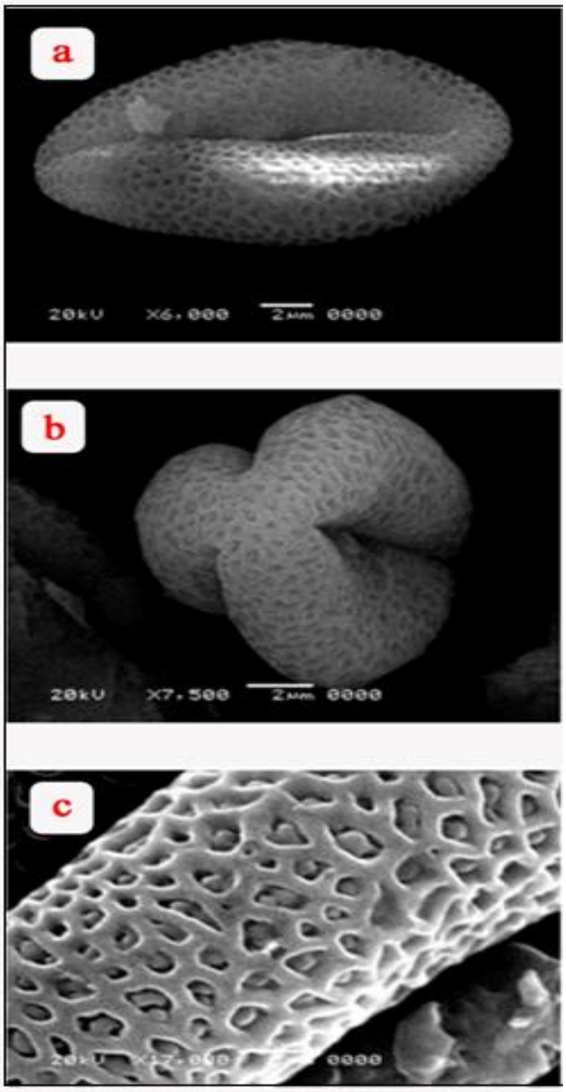

Fig. 7. SEM micrographs of *Ochradenus baccatus* inconstant male flower, Form 4 showing tricolpate pollen grains **(a):** equatorial view, **(b):** oblique polar view, **(c):** detail of ornamentation.

The sexual polymorphic pattern observed in *O. baccatus* populations in Egypt may be attributed to the polyploidy phenomenon (refer to the exclusion of external factors due to collection of all the studied populations from the same site). This phenomenon first noticed by Jennings (1976), Baker (1984) and highlighted by Miller and Venable (2000) who suggested that polyploidy can cause a shift towards uni-sexuality through the disruption of the self-incompatibility (SI) mechanism. Miller and Venable (2000) and Ashman *et al.* (2013) reported that "the transition from diploid hermaphrodites to polyploids with sexual dimorphism is the most common pattern in plants". The author's postulation was also supported by Martín-Bravo *et al.* (2007), who reported that the polyploidy acted as an important cytogenetic mechanism in the evolution of the Resedaceae, and by Mohamed (1997) who found the chromosome number of *O. baccatus* 2n= 56 in his study on specimens collected from Wadi Hof, Egypt. His results suggested an evolutionary increment of haploid number from the proposed basic chromosome number x=5 (González Aguilera and Fernández Peralta, 1984).

Finally, we can conclude that the sex plasticity and the male inconstancy aspects in *O. baccatus* needs further molecular studies to elucidate these morphs and evaluate the impact of the external and the internal factors on *O. baccatus* and other dioecious or subdioecious species especially in the arid environment to clarify the future evolution trends in such plant groups.

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