Infra-Specific Genetic Diversity of *Ochradenus Baccatus* Delile, a Gynodioecious Species in Egyptian Flora

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Abstract: *Ochradenus Delile is a small genus of 6-9 species within family Resedaceae. In Egypt, it is only represented by the gynodioecious Ochradenus baccatus Delile, the most widespread species across the entire range of the genus. Within Egypt, field populations and herbarium specimens of O.baccatus showed vast morphological and gender diversity within and between different populations even in the same habitat. This enhanced authors to apply additional genotypic information using RAPD technique to differentiate between the studied O.baccatus forms and populations to support the taxonomic identification of this species. The morphological examination revealed the presence of four transitional inconstant male forms (MF1-MF4) and three female forms (FF1-FF3) on the basis of their floral and fruit traits.The prescreening of the studied forms using twenty primers resulted in 109 scorable bands of which 50 (45.9 %) were polymorphic. All the primers used developed monomorphic bands with the studied O.baccatus forms ranged from 3 in OPZ-13 primer to 6 in OPD-05 primer. This study revealed the detection of certain RAPD bands characterized and linked to different sex forms. The range of Nei's genetic diversity among the studied forms appeared to be relatively limited (0.05-0.25). The UPGMA dendrogram obtained from RAPD results fit the calculated genetic distance and allowed two main groups to be distiguished. Group 1 included male forms and Group 2 included female forms. The results of this study suggested that the evolution of dioecy is associated with a significant decline in the amount of genetic diversity, and confirmed the presence of internal (genetic) factors controlling formation of the sex forms in the gynodioecious Ochradenus baccatus in Egypt not environmental factors.*

Keywords: *Ochradenus baccatus, gynodioecy, inconstant male, RAPD, genetic diversity, evolution, Egyptian Flora.*

1. INTRODUCTION

The genus *Ochradenus* (Brassicales, Resedaceae; [1])has been traditionally classified within the tribe Resedeae, which is characterized by syncarpic ovary, numerous ovules on parietal placentation, hypogenous flowers and presence of three carpels [2] and [3]. It is a small genus of 6-9 species dependent on different taxonomical treatments proposed [4], [5] and [6]. It is widely distributed in arid regions (deserts and steppes) of Africa (N, NE Tropical) and Asia (Temperate and Tropical), with South Arabian Peninsula as the center of its diversity [4], [5] and [7]. Members of *Ochradenus* are typically spinescent, intricately branching switch shrubs, with deciduous leaves, and frequently polygamous flowers with absent or ephemeral corolla. These features have been interpreted as adaptations to extremely xeric environments [8] and [9].However, [6] declared that reduction in size or loss of petals and polygamous flowers are considered as a transitional state in the evolutionary pathway from hermaphroditism to dioecy.

In Egypt, the genus *Ochradenus* is only represented by *O.baccatus* Delile [10]. It is the most widespread species across the entire range of the genus.It is distinguished by its white, baccate fruits at maturity and frequently minutely papillose seeds.

Previous studies considered this species to be dioecious [11] and [4]; while [12] indicated that populations of *O. baccatus* is gynodioecious, with females (constant in sex expression) and inconstant males (exhibit great variation in functional gender).

According to [13], females arise in hermaphroditic populations through spontaneous male sterility mutations. In a few species, sexuality has been shown to be influenced by environmental changes, but for the vast majority of gynodioecious species, male sterility is genetically determined. Genetic male sterility is widespread among flowering plants and have been documented in 617 plant species. Sex expression may be controlled by nuclear genes, cytoplasmic genes, or by cytoplasmic-nuclear gene

interactions. The majority of naturally occurring gynodioecious species have cytoplasmic-nuclear sex determination [14], [15] and [16].

Studies of natural populations of gynodioecious species have revealed that both CMS (cytoplasmic male sterility) and restorer loci often display a high degree of polymorphism [15] and [17]. In the few studies in which the genetic compositions of several populations of the same species have been compared, it was also found that local populations can differ markedly with regard to allelic frequencies at the sex-determining loci [18], [19] and [20].

The utility of PCR-based RAPD as phylogenetic marker for investigating evolutionary relationships among plants has been clearly established [21-28]. [29] analyzed genetic similarity in some wild species and cultivars of *Dianthus caryophyllus* (carnation) using isozyme and RAPD markers and showed that these markers can distinguish the cultivars and wild species of carnation.

Although, several molecular studies have been conducted in the order Brassicales, little attention has been paid to the origin and internal phylogenetic relationships of the Resedaceae[6].[30] and [31] performed an extensive survey of the phylogenetic relationships within core Brassicales, based on plastid sequence data from various molecular markers (*matK*, *ndhF* and *rbcL*).They suggested for the first time monophyly of Resedaceae. Molecular phylogenies were reconstructed for Resedaceae by [6] based on nuclear ribosomal DNA (nrDNA) ITS and plastid *trnL-F* sequences. In their study, species diversity and endemism together with the distribution of the main lineages and their phylogenetic relationships were analysed to obtain biogeographic insights in Resedaceae. The results supported the phylogenetic placement of *Oligomeris*, *Ochradenus* and *Randonia* within genus *Reseda.*

On species level, [32] used the nr DNA-ITS and chloroplast spacer sequences (*rpoB*and *rpoC1*) and cited that this technique was highly informative in differentiating the two similar species *O. baccatus* and *O. arabicus* in Saudi Arabia. Genetic stability of synseed grown plants and mother plant of *Ochradenus baccatus* was evaluated by inter-simple sequence repeat (ISSR) marker [33]. In this study, the mother plant as well as regenerated plants from synseed resulted in a monomorphic banding pattern developed from ISSR markers confirming genetic stability among the clones and helping in multiplication and conservation of this plant for commercial use in Saudi Arabia.

As far as the authors know, there are no earlier morphological and molecular studies carried out on gynodioecious *O. baccatus* to assess the genetic diversity among the different sex morphs of this species in Egypt and elsewhere.

The field and herbarium specimens observations of *O. baccatus* showed vast morphological and gender diversity within and between different populations. This enhanced authors to apply additional genotypic information to differentiate between the studied *O. baccatus* forms and populations to support the taxonomic identification of the studied species and its population.

During this study, RAPD technique will be applied to different *O. baccatus* forms hopefully to evaluate one of the internal factors (genetic factor) that may be the cause of this diversity among representative *O. baccatus* populations in Egypt.

2. MATERIAL AND METHODS

2.1. Study Area

Wadi Degla lies in the northern part of the Eastern Desert in Egypt, between longitude 31° 19' to 37' and latitude 29° 53' to 57'. It is an ancient and steep limestone valley. In this wadi, the soil is mostly composed of rock waste varying in texture from silt to gravels and boulders. The bed of the wadi is covered with layers of differing textures, influencing water availability [34]. Aridity prevails throughout the area: low and irregular rainfall, the highest values of rainfall are 25.3 ± 14.23 mm, the highest temperature is 27.66 ± 1.2 °C and the lowest temperature is 16.63 ± 4.44 °C [35].

2.2. Plant Material

2.2.1. For Morphological Data

Data on morphological variation between different sex morphs of *Ochradenus baccatus* in Egypt were based on herbarium collections kept in CAI as well as observations during the fieldwork.

Species delimitation and aspects of infraspecific morphological variation were established following three taxonomical treatments [3], [4] and [5], with some modifications. Abbreviations of the author's names followed [36].

During 2-years period (2012-2014), a survey throughout the range of *O. baccatus* in Egypt was conducted to collect and determine different sex morphs. For the infraspecific individuals, aspects of floral and fruit variation were recorded comparatively in Table 1 and 2. In this study, plant material was from a population of 20 permanently marked individuals (8 females and 12 inconstant males) at wadi Degla. The male plants (inconstant male or pollen-producing plants) are represented by: MF1 (male form1), MF2 (male form 2), MF3 (male form 3) and MF4 (male form 4), (Fig. 1); while female plants are represented by: FF1: female form 1, FF2: female form 2 and FF3: female form 3 (Fig. 2).

2.2.2. For Molecular Data

Juvenile leaves from the different studied forms of *O. baccatus* were collected from wadi Degla (to nullify the ecological factor) and kept at-20° C for DNA isolation.

2.2.2.1. DNA isolation

Table3. *Sequence of primers used and number of polymorphic and monomorphic bands obtained for the studied forms of Ochradenus baccatus.*

Primer code	Sequence	Size (bp) min-	No. of poly-morphic	No. of mono-morphic
OPA-02	TGCCGAGCTG	340-1797	3	4
OPA-13	CAGCACCCAC	260-920	2	4
OPA-15	TTCCGAACCC	270-1140	4	4
OPB-18	CCACAGCAGT	310-1500	3	5
OPC-03	GGGGGTCTTT	450-1330	8	1
OPC-16	CACACTCCAG	370-1990	2	4
OPC-20	ACTTCGCCAC	300-1800	3	4
OPD-05	TGAGCGGACA	220-1500	3	6
OPE-10	CACCAGGTGA	340-1700	3	4
OPG-06	GTGCCTAACC	220-970	3	4
OPH-03	AGACGTCCAC	510-1500	3	3
OPK-12	TGGCCCTCAC	300-1700	3	4
OPN-08	ACCTCAGCTC	355-1750	7	4
OPP-13	GGAGTGCCTC	400-1500	3	5
OPZ-13	GACTAAGCCC	490-840	0	3

Seven DNA samples were extracted from 50 mg of juvenile leaf tissues in each sample according to [37] using the modified CTAB protocol. RNA was eliminated by adding 0.7 units of RNAase to each sample. The DNA was dissolved in 20 µl sterilized bi-distilled water. Polymorphism of DNA in each sample was detected using standard DNA primers (twenty primers were obtained from the Information Company Operon Technologies Inc., Almeda California). Sequences of the used primers were outlined in Table 3.

2.2.2.2. Polymerase Chain Reaction (PCR)

The DNA fraction purified from previous protocol was submitted to enzymatic amplification using an automatic thermal cycler. PCRs were conducted in a total volume of 50 µl in a Perkin Elmer Cetus DNA Tehermal Cycler. The agarose fragment containing the nucleic acid band was melted and 1 µl of the agarose-DNA suspension directly added to the PCR mixture. The reaction mixture (50µl) contained 16.6 mM $(NH_4)_2 SO_4$, 67 mMTris-HCL pH 8.8 (at 25°C), 6.7 mM $MgCl_2$,10 mMβ-mercaptoethanol, 200 µM each of dATP, dCTP, dGTP and dTTP, 0.05 % (v/v) detergent W-1 (Bethesda Research Laboratories, Githersburg, MD), 0.3 µg each of amplification primers, and 2.5 units Taq polymerase (Bethesda Research Laboratories, Githersburg, MD). The amplification reaction was performed in 500 µl Eppendorf vials using a Perkin Elmer-Cetus (Thermal Cycler; Perkin Elmer, Norwalk, USA). The amplification conditions were set at 95°C for 1 min (DNA denaturation),

55°Cfor 20 sec (oligonucleotide annealing) and 72°C for 15 sec (primer extension) for 30 cycles. The amplification was done with random synthetic-20-oligonucleotide primers of 10 bases each (Table3). The developed bands stained by ethidiumbromide, visualized under UV-light and photographed.

2.2.2.3. Statistical Analysis

RAPD-PCR-amplified fragments were scored as present (1) or absent (0), only clear bands were scored according to [38]. Nei's genetic distance [39] was calculated to examine the genetic diversity among the studied forms, data analyses were performed using SPSS version 17.0 (SPSS 2009).

The expected genetic distance ranged from 0 to 1 depending on the degree of similarity between the assessed RAPD profiles of the studied forms. Sequential Agglomerative Hierarchical Non-overlapping (SAHN) clustering using the unweighted pair group method with arithmetic mean (UPGMA) method was then performed and a dendrogram was generated as described by [40] using the numerical taxonomy and multivariate analysis system for the IBM PC (NTSYs), version 2.20U software [41].

3. RESULTS

3.1. Morphological Treatment

Ochradenus baccatus Delile, Hist. Nat. 2 (1): 63, tab. 31, fig. 1 (1813), [42].*Type*: Haute Égypte: "Medynet-abouQournah et Denderah, sur les limites du désert"; *Delile*s.n. (P, Holotype).

Glabrous, gynodioecious shrubs, 0.5-2 (-3) m high. Shoots much branched; branches green, turning yellowish-brown sulcate when dry, terete, erect to ascending or intricate, divaricate at the spinescent tip. Leaves sessile, alternate, with a yellow glandular swelling at the base, laterally with 2 basal dents, single or fascicled (2-4); laminalanceolate-linear to narrowly oblanceolate, 5-50 mm long, 0.5-2.0 (-6) mm in diam., gradually or abruptly tapering above into an acute or acuminate apex, entire, glabrous, caducous. Inflorescence terminal, racemose, spikate, erect to ascendant, solitary or rarely branched, glabrous throughout; racemes bracteate, shortly peduncled, varying from narrow slender, with more or less lax flowers to broadly cylindrical or pyramidal, with densely packed flowers, often thorn-tipped later. The investigated *O. baccatus* forms were sorted on the basis of their floral and fruit traits.
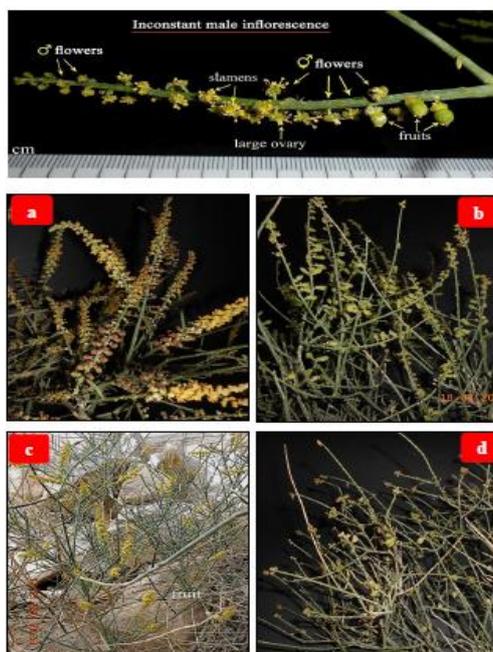
Figure1. *The inconstant male forms; (a): Form 1, (b): Form 2, (c): Form 3, (d): Form 4.*

Staminate racemes (Inconstant male forms, Fig.1): Four transitional inconstant male forms (MF1-MF4) were recognized, the major differences were outlined in Table (1). Peduncle 2-10 mm long; flowers commonly dense, with male and hermaphrodite flowers at varying frequencies, upper male, lower hermaphrodite. In male and hermaphrodite flowers: flowers yellow, shortly pedicelled; pedicel glabrous; bracts caducous, fleshy, frequently concave, lanceolate-oblong, acute. Calyx of 5-6 free sepals; sepals persistent, unequal, the longest narrowly oblong to linear, 0.7-1.5 mm long, obtuse to

subacute, the shortest ovoid, 0.5-0.6 mm long, acute. Petals absent. Disc single, oblique, orbicular, fleshy reflexed, glabrous, with uneven undulate margin. Stamens (7-) 10-13, unequal; anthers oblong or ovoid, 0.5-1.0 x 0.5-0.7 mm, glabrous, smooth, cordate at the base; filaments caducous, glabrous, subulate, fused at the base and attached to the disc. In male flowers, pistils rudimentary, less than 1 mm long; in hermaphrodite flowers, pistils ovoid-cylindric, rarely obovoid with flattened base, 1.0-2.5 mm long, slightly constricted above, with 3 apical teeth; teeth short, erect, blunt, with unequal stigmata at apex; ovules2-8 ovules / placenta, in 2 rows. Fruit berry-like, green tinged with red, white and transparent at maturity, ovoid or obovoid to globose or ellipsoid, with reddish-brown stigmata; stigmata unequal and recurved. Seeds yellow or brown to black, reniform in outline, ovoid, dull, with narrow, closed sinus; testa minutely papillose.

Table1. *Floral and fruit traits within various forms of inconstant male (MF).*

Character	Form (1)	Form (2)	Form (3)	Form (4)
● Inflorescence shape	dense, cylindrical to pyramidal.	dense, cylindrical.	dense, cylindrical.	lax or dense, narrowly slender or cylindrical.
● Inflorescence length	≤ 20 cm	≤ 30 cm	≤ 30 cm	≤ 30 cm
● Inflorescence width	1-2 cm	1-2 cm	1-2 cm	< 1 cm
● Flowers no. 40-80	+	up to 100	up to 100	up to 180
● Bract length	< 2 mm	< 3 mm	< 3 mm	≤ 2 mm
● Pedicel length	≤ 1 mm	≤ 2 mm	≤ 2 mm	≤ 2 mm
● Bract / pedicel	1-2	2-3	2-3	1-1.5
● Sepals no.	6	6	6	5 or 6
● Flowers diam.	≤ 6 mm	≤ 4 mm	≤ 4 mm	≤ 6 mm
● Stamens no.	(8-)10-13	(7-)10-13	(7-)10-13	(8-)10-13
● Stamens with long erect filaments.	2-3	1-3	1-2	1-4
● Disc diam.	≤ 3 mm	≤ 2.5 mm	≤ 2.5 mm	≤ 4 mm
● Ovary length	≤ 2.5 mm	≤ 2.5 mm	≤ 2.5 mm	< 1 mm
● Ovary width	≤ 2 mm	≤ 1.5 mm	≤ 1.5 mm	< 1 mm
● No. ovules /placenta	≤ 8	≤ 5	≤ 5	≤ 5
● No. ovules / ovary	> 14	≤ 14	≤ 14	< 14
● Fruit length	up to 7 mm	up to 7 mm	up to 7 mm	up to 5 mm
● Fruit width	≤ 6 mm	≤ 5 mm	≤ 5 mm	≤ 4 mm
● % fruit productivity	50-80 %	20-30 %	1-8 %	0.003 %
● No. of seeds / fruit	≤ 11	≤ 11	≤ 11	< 10
● No. of undeveloped seeds	< 10	≤ 10	≤ 10	< 10

Pistillate racemes: (Female forms, Fig.2): Three female forms (FF1, FF2 and FF3) were recognized, the major differences were outlined in Table (2). Racemes are generally longer than staminate ones; peduncle 2-10 mm long. Flowers 1-3 mm in diam., shortly pedicelled; bract, pedicel, calyx, petals and stigmata as in staminate racemes. Stamens 10-13, vestigial, 0.5 mm long, with sterile anthers; anthers oblong. Pistils ovoid-cylindric, 1-2 mm long, 1.0-1.5 mm in diam. Ovules 2-7 / placenta, in 2 rows,. Infructescence is either long, narrow, slender and lax (FF1), or short, pyramidal with dense fruits conferted at the apex and base (FF2), or short, cylindrical with dense fruits conferted at the base and deciduous above (FF3). Fruit ovoid or obovoid to globose. Seeds as in staminate racemes; testa commonly minutely papillose, rarely smooth.

Table2. *Floral and fruit traits within female forms (FF).*

Character	Form (1)	Form (2)	Form (3)
• Inflorescence shape	long, lax, narrow slender.	short, dense, pyramidal or cylindric, conferted at the apex and base.	short, dense, cylindric, conferted at the base and deciduous above.
• Inflorescence length	≤ 45 cm	≤ 20 cm	≤ 20 cm
• No. of flowers	≥ 70	< 70	< 70
• Disc diam.	2-3 mm	1-2 mm	1-2 mm
• No. of ovules / placenta	≤ 7	≤ 4	≤ 4
• No. of ovules / ovary	10-15	<10	<10
• % fruit productivity	60-85 %	50-85 %	30-70 %
• No. of seeds / fruit	10-15	< 10	< 10
• No. of undeveloped seeds	―	1-3	1-2

Figure2. *The studied female forms*

3.2. Molecular Analysis
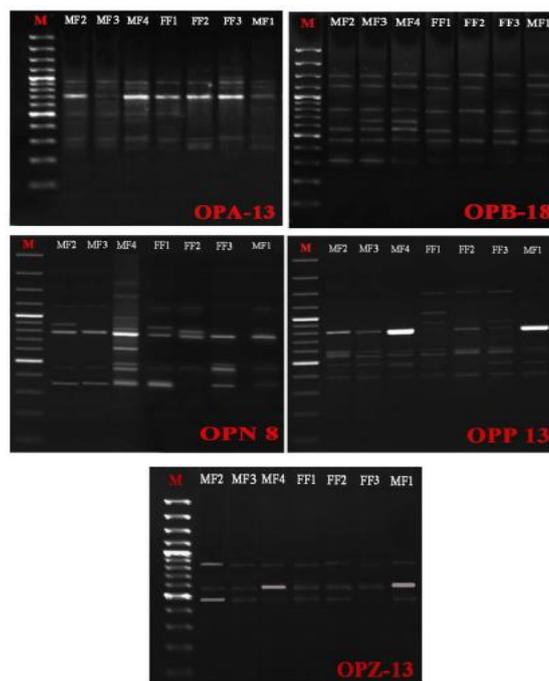
Figure3. *Gel electrophoresis of DNA amplification products obtained with five representative RAPD primers (out of fifteen) in the studied forms of Ochradenus baccatus: MF1-4: inconstant male forms; FF1-3: female forms and M: 100 bp marker*

From the tested twenty RAPD primers, fifteen showed positive results. Some of these primers showed high resolution bands in relation to the number of base pairs as in primer OPN-08 (11 bands), while others showed little alignment to the tested *Ochradenus baccatus* forms as in primer OPZ-13 which developed only 3 bands. The prescreening of the studied forms resulted in 109 scorable bands of which 50 (45.9 %) were polymorphic (Table 3). The number of polymorphic bands per primer ranged from 0 (in OPZ-13 primer) to 8 (in OPC-03 primer). All the primers used developed monomorphic bands with the studied *O. baccatus* forms ranged from 3 (in OPZ-13 primer, 100 % monomorphic bands, Fig. 3) to 6 (in OPD-05 primer). Among the used primers, OPC-03 produced most of the polymorphic bands.

The developed monomorphic bands appeared similar in all the studied *Ochradenus baccatus* forms (Fig.3). Among the characteristic monomorphic bands were: at 1797, 1254, 611 and 340 bp for OPA-02; at 920, 700, 310 and 260 bp for OPA-13; at 900, 580, 450 and 270 bp for OPA-15; at 1500, 1260, 800, 483 and 310 bp for OPB-18; at 1330 bp for OPC-03.

The inconstant male form 1 (MF1) was characterized by its unique developed bands at 1100 bp for OPB-18, 995 bp for OPC-03, 950 bp for OPD-05 and at 900 bp for OPH-03. The most interesting finds were the presence of female-linked bands, among them: bands at 1100, 590 and 450 bp for OPC-03, at 1800 bp for OPC-20, at 1700 bp for OPK-12, at 1900 and 950 bp for OPP-13. While, the bands at 760 bp for OPA-02, at 1000 and 850 bp for OPA-15, at 860 bp for OPE-10 were characteristic to the studied inconstant male forms or male-linked bands.

The retrieved bands showed a genetic diversity among the studied female forms, among them the developed bands at 1600 bp for OPA-02, at 500 bp for OPA-13, at 1600 bp for OPE-10, at 520 bp for OPG-06 and at 1100 bp for OPP-13 characterized the female form 1 (FF1) from the other female forms. While, the band at 950 bp for OPN-08 can be used to differentiate the female form 2 (FF2) from the other female forms. Similarly, the bands at 850 bp for OPA-13, at 680 bp for OPC-03, at 300 bp for OPC-20, at 300 bp for OPK-12 and at 500 bp for OPN-08 can differentiate the female form 3 (FF3) from the others.

A notable genetic diversity was also observed among the studied male forms, the developed band at 700 bp for OPA-15 characterized the inconstant male form 2 (MF2) from the other male forms. While, for the primer OPN-08, the developed bands at 950, 500bp were characteristic to form 4 (MF4) from the other male forms and the bands at 1750, 1400, 1000 and 600bp were unique to this form out of the remaining studied forms.

3.3. Genetic Variation

The genetic distance between the studied *Ochradenus baccatus* forms based on Nei's index ranged from 0.05 between inconstant male forms (MF2 & MF3) to 0.25 between inconstant male form 4 (MF4) and female form 1 (FF1), (Table 4). Among female forms, the genetic distance ranged from 0.08-0.09, while it ranged from 0.05-0.15 among male forms. The UPGMA dendrogram (Fig.4) obtained from RAPD results fit the calculated genetic distance and allowed two main groups to be distinguished. Group 1 included male forms and Group 2 included female forms.

Table4. *Genetic distance between the studied Ochradenus baccatus forms based on Nei's Index.*

Forms	MF1	MF2	MF3	MF4	FF1	FF2	FF3
MF1		0.15	0.06	0.10	0.19	0.21	0.13
MF2	0.15		0.05	0.09	0.13	0.17	0.11
MF3	0.06	0.05		0.07	0.16	0.21	0.15
MF4	0.10	0.09	0.07		0.25	0.18	0.20
FF1	0.19	0.13	0.16	0.25		0.09	0.08
FF2	0.21	0.17	0.21	0.18	0.09		0.08
FF3	0.13	0.11	0.15	0.20	0.08	0.08	
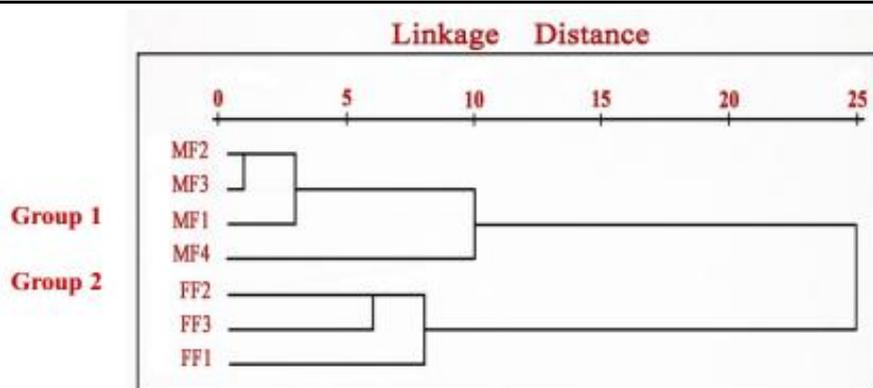
Figure4. *UPGMA dendrogram of the studied Ochradenus baccatus forms based on Nei's genetic distance.*

4. DISCUSSION

Considerable progress has been made in our understanding of the evolutionary lability and function of plant sexual traits through imaginative experiments on floral characters and through the use of genetic markers. In addition, molecular studies have aided in the reconstruction of the phylogenetic history of sexual systems and have provided new information on the consequences of mating-system variation for genetic diversity [43].

In recent years, attempts have been made to understand the genetic basis of the sex determination in plants, which can make the use of molecular markers viz., RAPD, ISSR, AFLP to identify the sex-linked DNA marker in plants. RAPD technique was recommended by [44], who claimed that RAPDs overestimated the genetic similarity when compared to RFLP.

This study provides the first detailed analysis of genetic variability of morphologically variable *Ochradenus baccatus* population forms in Egypt. The morphological treatment revealed the importance of the floral (bract, pedicel, stamens,…Table 1 & 2) and fruit characters (% fruit productivity, no. of seeds/ placenta,…Table 1 & 2) in differentiation between forms. The most important character was the % of fruit productivity which varied greatly among inconstant male forms (ranged from 50-85 % in form 1 to 0.003 % in form 4). The traced morphological differences among the studied population sex forms were traced earlier by [12] on *O. baccatus* from Israel.

In the molecular study, RAPD markers revealed 45.9% of the total polymorphic loci among the studied *Ochradenus baccatus* population forms, as shown in Table (3). The constructed dendrogram showed the close affinity between male forms together as one group (Group 1; with genetic distance ranges from 0.05-0.15) and female forms together as one group (Group 2; with genetic distance ranges from 0.08-0.09; Fig. 4& Table 4). The results revealed that the female specific DNA fragments of size 954, 590 and 450 bp produced by the primer OPC-03, at 1800 bp for OPC-20, at 1700 bp for OPK-12, at 1900 and 950 bp for OPP-13 were tightly linked with the female sex locus and were useful for sex determination of the studied sex forms. While, the developed bands at 760 bp for OPA-02, at 1000 and 850 bp for OPA-15, at 860 bp for OPE-10 characterized the male forms from the female forms. These results were supported by [45] in their study on *Carica papaya,* who have found OPB-01 was male specific and OPB-05 was female specific marker. Also by [46] in their application of RAPD on the polygamodioecious plant *Simarouba glauca* to determine the sex specific molecular marker using RAPD and SCAR markers and found one primer associated with maleness in *S. glauca*.

The developed bands at 950, 500 bp for the primer OPN-08 (Fig. 3) differentiated the inconstant male form 4 (MF4) from the other male forms. This result agreed with our postulation of the increase in male fitness in MF4 (with fruit production 0.003%). While, the presence of 805 bp band for OPC-03 and bands at 1200, 570 bp for OPC-16 in inconstant male form 2 (MF2) and inconstant male form 3 (MF3) along with the inconstant male form 1 (MF1) and their absence in MF4, indicated that these two forms (MF2 & MF3) must be in transitory stage of male from form 1 to form 4.

These achieved results by RAPD application were confirmed by earlier studies carried out for detection of sex linked RAPD markers in several dioecious species. Among of them, [47] who identified thirty two male-specific RAPD bands in hop (*Humuluslupulus* L.) and [48] who found RAPD fragment of 400 bp size closely linked with the male sex type of hemp (*Cannabis sativa* L.).

The pointed gourd (*Trichosanthesdioica* Roxb.) has also been studied and found to have a RAPD marker associated with females that was absent in all male plants [49]. Similarly, the presence of a female-specific band in nutmeg (*Myristicafragrans* Houtt.) has also been reported by [50] using the screening of RAPD primers.

The range of Nei's genetic diversity among the studied forms appeared to be relatively limited (0.05-0.25). Our findings do not represent an isolated case, since other dioecious species varying in life and/or evolutionary history also show reduced genetic diversity when compared with non-dioecious species [51-57]. In Egypt, to our knowledge, the approach adopted in this study represents the first study to compare the genetic structure of morphologically diversed forms of a gynodioecious species.

Our results concludes that the evolution of dioecy was associated with a significant decline in the amount of genetic diversity. This conclusion was supported by earlier works of [55], [56] and [57].Application of RAPD technique in this study confirmed the presence of internal (genetic) factors controlling formation of the sex forms in the gynodioecious *Ochradenus baccatus* in Egypt, and this is not related to environmental factors as mentioned by [12]. This phenomenon still requires further work involving multiple phylogenetically independent comparisons.

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