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TAXONOMIC RELATIONSHIPS OF SOME SPECIES OF *OROBANCHE* L. EVIDENCE FROM RAPD-PCR AND ISSR MARKERS

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Abstract

The taxonomic relationships among 25 samples representing nine species of *Orobanche* L. (Orobanchaceae) were determined by the analysis of morphological characters and molecular polymorphism using random amplified polymorphic DNA (RAPD) and inter simple sequence repeat (ISSR). In order to construct dendrogram elucidating the relationships among the examined taxa, the coded data were analyzed using the software package NTSYS-pc 2.1 based on the Neighbor-joining (NJ) tree building method based on a distance matrix. The aim of this study is to develop taxonomic relationship based on morphological and molecular data, in order to obtain a more reliable taxonomic relationship of *Orobanche* species under study. The dendrogram produced by the analysis of the molecular data (RAPD and ISSR) resembled that constructed by NJ dendrogram for the morphological variation. The studied taxa were separated in two groups, the first comprised of the five species of section *Trionychon* (*O. purpurea, O.lavandulacea, O. ramosa, O. mutelii* and *O. aegyptiaca*) and the second comprised of the four species of section *Orobanche* (*O.cernua, O. crenata, O. minor* and *O. pubescens*). High similarity was detected between *O. pubescens* and *O. minor*. The results confirmed the close relationship between *O. ramosa* and *O. mutelii*. Moreover, this study demonstrated the grouping of the studied taxa in most cases by geographically isolated population.

Key words: ISSR, Morphology, *Orobanche*, RAPD- PCR, Taxonomy, *Trionychon*.

Introduction

Orobanche L. is a genus of holophrastic flowering plant that has lost its autotrophic properties in favor of a parasitic lifestyle (Cronquist, 1988). It belongs to the family Orobanchaceae which comprises 170-200 species of parasitic herbaceous plants mostly native to the temperate Northern Hemisphere (Beck-Mannagetta, 1890; Uhlich *et al*., 1995). Among the *Orobanche* species, *O. crenat*a, *O. cumana*, *O. ramosa*, *O. cernua* and *O. mutelii* were recognized as parasitic plants of economically important crops (Katzir *et al*., 1996; Paran *et al*., 1997). Comparative studies regarding *Orobanche* species from different natural habitats are of great importance since they can clarify the evolutionary path from being non weedy parasitic plants to aggressive weedy parasitic weeds (Verkleij & Pieterse, 1994).

Orobanche L. includes four sections: *Gymnocaulis* Nuttall, *Myzorrhiza* (Phil.) G. Beck, *Trionychon* Wallr and *Orobanche* L. (Beck-Mannagetta, 1930). In previous taxonomic treatments, these sections are recognized as separate genera: *Aphyllon* Mitchell, *Myzorrhiza* (Phil.) G. Beck, *Phelipanche* Pomel and *Orobanche* L. respectively (Holub, 1977, 1990; Teryokhin *et al*., 1993). Teryokhin (1997) classified *Orobanche*, *Phelypaea* and *Cistanche* in subtribe Orobanchinae, while *Phelipanche* constitutes the subtribe Phelipanchinae, implying that *Orobanche* is not monophyletic. Nuclear ITS, karyological and genome size data indicate that *Phelypaea* and *Orobanche* sections are closely related (both share a chromosome base number of ×=19), while the remaining sections (all with chromosome base number ×=12) constitute a separate lineage (Schneeweiss *et al*., 2004a, 2004b).

A number of recent studies have addressed the relationships among *Orobanche* species based on morphological variations (Andary, 1994; Román *et al*., 2003; Mohamed & Musselman, 2008). *Orobanche* and *Phelipanche* species are characterized by variation in their bracts, placentation type, inflorescence type, chromosome number and distribution. The molecular taxonomy of most economically important *Orobanche* species were analyzed with DNA markers, Zaid *et al*. (1997) used RAPD to investigated *O. aegyptiaca, O. oxyloba, O. ramose* and three *O. crenata* collections, gathered from different locations in Egypt, by using RAPD analysis. Benharrat *et al*. (2002) applied the ISSR for taxonomic studies of *Orobanchaceae* by using five different ISSR primers. Their results showed that, ISSR technique proved to be a good tool for distinguishing between closely related broomrape species (Benharrat *et al*., 2002). In addition, this technique was used to distinguish between different populations of *Orobanche crenata* (Román *et al*., 2002) and *Phelipanche ramosa* (Buschmann *et al*., 2005).

Thorogood *et al*. (2008, 2009a, 2009b), studied *O. minor* using ISSR and sequence-characterized amplified region (SCAR) markers. Their results demonstrated that *O. minor* was comprised of genetically divergent populations associated with different hosts in UK. Recently, one hundred ISSR primers were tested on specimens from five different species (*Phelipanche ramosa, P. mutelii, P. purpurea, Orobanche alba* and *O. minor*). Thirteen primers were found to produce polymorphic bands suitable to distinguish the known sections and genera. Another three primers could distinguish the genera and probably higher taxonomic ranks (Hristova *et al*., 2011).

The aim of this study was to determine the genetic relationships among *Orobanche* species from Egypt and Saudi Arabia, to evaluate the usefulness of the ISSR and RAPD technique in generating DNA markers for genetic

and taxonomic studies, and to relate morphological and molecular data, in order to obtain a better understanding of the taxonomic relationship of *Orobanche* species under investigation. The influence of the geographical distribution and the hosts' types on the relationship between the samples of the same species were also studied in the present investigation.

Materials and Methods

Materials: In the present study, 25 samples of *Orobanche* L. were collected from different localities representing two sections and nine species. The list of the studied taxa and their localities are given in Table 1.

Methods

Morphological investigation: A total of 49 morphological characters were described (Table 2). The macromorphological characters of the whole plant *viz*. habit, stem, leaf, flowers, inflorescence, capsule and seeds were described directly from the fresh specimens of the taxa under investigation.

DNA extraction and RAPD fingerprinting: For DNA extraction, seeds of 25 samples of the studied taxa were germinated at 20ºC for 15-20 days. Seedlings were collected on ice and DNA was extracted using the CTAB method based on Saghai-Maroof *et al*. (1984) as described in Badr *et al*. (2012). Twenty-five arbitrary 10-mer random primers (Operon Technologies, Inc., USA) were used for RAPD finger printing. However, only 10 primers gave clearly defined products (Table 3). PCR was carried out using a Biocycler TC-S thermal cycler from HVD, Austria. The PCR reactions were developed in a total volume of 50 µl with the following components: 5 µl of 10x reaction buffer (75 mM Tris HCl, pH 9.0, 50 mM KCl, 20 nM $(NH4)_2$ SO_4 and 0.001% bovine serum albumin), 2 µl of 25 mM of each primer, 1 µl of Taq DNA polymerase (1 U/µl), and 50mg/ µl template DNA. The volume was completed to 50 µl with deionized diethylpyrocarbonate (DEPC) and water. The following PCR program was used: an initial denaturation of DNA was carried out at 94ºC for 1 min, annealing at 37ºC for 1 min, extension at 72ºC for 2 min and a final extension at 72ºC for 12 min. The RAPD products were separated using 1.4% agarose gel in TAE buffer (0.04 M Tris-acetate, 1 mM EDTA pH= 8) by electrophoresis at 100 volt for 60 min. The gels were stained in 0.2 µg/ml ethidium bromide and photographed using a gel documentation system (Gel Doc-BioRad, 2000).

Table 1. A list of the studied taxa of the *Orobanche* species and their localities.

No.	Taxa	Locality
	I- Section: *Orobanche* L.	
1.	*O. cernua* Loefl. 1	On *Lycium showii*, Sinai, Egypt
2.	*O. cernua* Loefl. 2	On *Helianthus annus*, El- Arish, Egypt
3.	*O. cernua* Loefl. 3	On *Launaea lanifera*, Hail, Saudi Arabia
4.	*O. crenata* Forssk. 1	On *Trifolium alexandrinum*, Cairo, Egypt
5.	*O. crenata* Forssk. 2	On *Trifolium alexandrinum*, Sharquia, Egypt
6.	*O. minor* Smith. 1	On *Vicia faba*, Cairo, Egypt
7.	*O. minor* Smith. 2	On *Solanum tuberosum*, Hail, Saudi Arabia
8.	*O. minor* Smith. 3	On *Lycopersicum esculentum*, Hail, Saudi Arabia
9.	*O. pubescens* Urv. 1	On *Tropaeolum majus*, Cairo, Egypt
10.	*O. pubescens* Urv. 2	On *Tropaeolum majus*, Alexandria, Egypt
11.	*O. pubescens* Urv. 3	On *Tropaeolum majus*, Hail, Saudi Arabia
12.	*O. pubescens* Urv. 4	On *Anthemis pseudocotula*, Hail, Saudi Arabia
	II- Section: *Trionychon* Wallr	
13.	*O. aegyptiaca* Pers. 1	On *Lycopersicum esculentum*, Sharquia, Egypt
14.	*O. aegyptiaca* Pers. 2	On *Lycopersicum esculentum*, Alexandria, Egypt
15.	*O. aegyptiaca* Pers. 3	On *Lycopersicum esculentum*, Hail, Saudi Arabia
16.	*O. aegyptiaca* Pers. 4	On *Brassica oleraceae* spp. *botrytis*, Hail, Saudi Arabia
17.	*O. lavandulaceae* Reichenb.	On *Cucurbita pepo*, Sharquia, Egypt
18.	*O. mutelli* F.G. Schultz. 1	On *Medicago* sp. Alexandria, Egypt
19.	*O. mutelli* F.G. Schultz. 2	On *Malva parviflora*, Hail, Saudi Arabia
20.	*O. mutelli* F.G. Schultz. 3	On *Rumex vesicarius*, Hail, Saudi Arabia
21.	*O. purpurea* Jacq. 1	On *Artemisia judaica*, Hail, Saudi Arabia
22.	*O. purpurea* Jacq. 2	On *Artemisia monosperma*, Hail, Saudi Arabia
23.	*O. ramosa* L. 1	On *Lycopersicum esculentum*, Sharquia, Egypt
24.	*O. ramosa* L. 2	On *Trifolium alexandrinum*, Sharquia, Egypt
25.	*O. ramosa* L. 3	On *Trifolium alexandrinum*, Alexandria, Egypt

Table 2. Macromorphological characters of the whole plant of the studied taxa. Taxa are arranged according to their numbers in table 1.

No	Character	State	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
1.	Habit	Annual Perennial	P	P	P	A	A	A	A	A	A	A	A	A	P	P	P	P	P	P	P	P	P	P	P	P	P
2.	Parasitic on	Cultivated plants Wild plants	W	C	W	C	C	C	C	C	C	C	C	C	C	C	C	C	C	W	W	W	W	W	C	C	C
3.	Stem	Unbrached Branched	U	U	U	U	U	U	U	U	U	U	U	U	B	B	B	B	B	B	B	B	B	B	B	B	B
4.	Stem texture	Glabrous Tomentose Villous Glandular pubescent	G	G	G	P	P	T	T	T	T	T	T	T	T	T	T	T	Gl	T	T	T	P	P	V	V	V
5.	Stem cross section	Cylindrical Angular Irregular	I	I	I	C	C	C	C	C	C	C	C	C	C	C	C	C	C	A	A	A	I	I	A	A	A
6.	Leaf (Scales) duration	Leafless Leafy	L	L	L	Ls	Ls	Ls	Ls	Ls	Ls	Ls	Ls	Ls	L	L	L	L	Ls	Ls	Ls	Ls	L	L	Ls	Ls	Ls
7.	Leaf (scales) arrangement	Spirally Imbricate	S	S	S	-	-	-	-	-	-	-	-	-	S	S	S	S	-	-	-	-	I	I	-	-	-
8.	Leaf shape	Ovate Ovate-lanceolate Lanceolate	L	L	L	-	-	-	-	-	-	-	-	-	OL	OL	OL	OL	-	-	-	-	O	O	-	-	-
9.	Leaf texture	Tomentose Glandular	G	G	G	-	-	-	-	-	-	-	-	-	T	T	T	T	-	-	-	-	V	V	-	-	-
10.	Leaf apex	Obtuse Acute	A	A	A	-	-	-	-	-	-	-	-	-	O	O	O	O	-	-	-	-	O	O	-	-	-
11.	Pedicel hairs	Absent Scattered Dense	A	A	A	-	-	-	-	-	-	-	-	-	S	S	S	S	S	S	S	S	D	D	A	A	A
12.	Inflorescence type	Spike Raceme	S	S	S	S	S	S	S	S	S	S	S	S	R	R	R	R	R	R	R	R	R	R	R	R	R
13.	Inflorescence structure	Loose Dense	D	D	D	D	D	L	L	L	L	L	L	L	L	L	L	L	D	D	D	D	D	D	L	L	L
14.	Bract shape	Ovate Triangular Lanceolate	L	L	L	L	L	O	O	O	T	T	T	T	L	L	L	L	T	L	L	L	O	O	T	T	T
15.	Bract size	Shorter than calyx Equal Calyx	E	E	E	E	E	E	E	E	E	E	E	E	S	S	S	S	S	S	S	S	S	S	S	S	S
16.	Bract length	030-0.40 cm **(1)** > 0.40 cm **(2)**	2	2	1	2	2	2	2	2	2	2	2	2	2	2	2	2	1	1	1	1	1	1	1	1	1

Table 2. (Cont'd.).

No	Character	State	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
17.	Bracteoles	Absent Two	A	A	A	A	A	T	T	T	A	A	A	A	T	T	T	T	T	T	T	T	T	T	T	T	T
18.	Calyx shape	Cupular Campanulate	Ca	Ca	Ca	C	C	Ca	Ca	Ca	Ca	Ca	Ca	Ca	Ca	Ca	Ca	Ca	C	C	C	C	Ca	Ca	C	C	C
19.	Calyx length	0.40-0.80 cm **(1)** > 0.80 cm **(2)**	2	2	2	2	2	2	2	1	2	2	2	2	2	2	2	2	2	2	2	1	2	2	1	1	1
20.	Calyx apex	**2**-lobed **4**-lobed **5**-lobed **6**-lobed	2	2	2	2	2	5	5	5	5	5	5	5	4	4	4	4	2	4	4	4	6	6	5	5	5
21.	Calyx lobe shape	Lanceolate Triangular	T	T	T	T	T	T	T	T	T	T	T	T	L	L	L	L	L	L	L	L	L	L	L	L	L
22.	Calyx lobe tip	Entire Toothed	T	T	T	E	E	T	T	T	T	T	T	T	E	E	E	E	E	E	E	E	E	E	L	L	L
23.	Corolla colour	White Yellow Blue Violet	B	B	B	W	W	V	W	W	W	W	W	W	B	B	B	B	W	V	V	V	W	W	Y	Y	V
24.	Corolla length	0.80 –1.00 cm **(1)** 1.10-1.50 cm **(2)** > 1.50 cm **(3)**	3	3	2	2	2	3	2	1	3	3	2	2	3	3	2	2	3	3	3	2	3	3	2	2	2
25.	Corolla shape	Campanulate Bilabiate	B	B	B	C	C	B	B	B	C	C	C	C	B	B	B	B	B	C	C	C	B	B	B	B	B
26.	Corolla	Straight Curved	C	C	C	C	C	C	C	C	S	S	S	S	S	S	S	S	C	S	S	S	S	S	C	C	C
27.	Corolla upper lobe	Entire **2**-lobed	2	2	2	E	E	2	2	2	E	E	E	E	2	2	2	2	2	E	E	E	2	2	E	E	E
28.	Corolla lower lobe	Entire **3**-lobed	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	E	E	E	3	3	E	E	E
29.	Corolla lobes length	Equal Lower lobe shorter than upper Lower lobe longer than upper	Ll	Ll	Ll	E	E	Ls	Ls	Ls	E	E	E	E	Ll	Ll	Ll	Ll	Ls	E	E	E	Ls	Ls	E	E	E
30.	Corolla lobe tip	Entire Toothed	T	T	T	E	E	T	T	T	E	E	E	E	E	E	E	E	E	E	E	E	T	T	E	E	E
31.	Corolla upper part	Whitish to pale blue Dark blue to violet Brown	W	W	W	D	D	D	D	D	D	D	W	W	W	W	W	W	B	D	D	D	B	B	D	D	D

Table 2. (Cont'd.).

No	Character	State	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
32.	Corolla dorsal line	**G**eniculate **I**nflated	G	G	G	G	G	G	G	G	G	G	G	G	I	I	I	I	I	I	I	I	I	I	I	I	I
33.	Anther	**G**labrous **V**illous	G	G	G	V	V	V	V	V	G	G	G	G	V	V	V	V	G	V	V	V	G	G	V	V	V
34.	Anther length	0.20-0.30 cm **(1)** 0.31-0.40 cm **(2)** > 0.40 cm **(3)**	3	3	3	2	2	1	1	1	3	3	2	2	3	2	2	3	3	2	2	2	3	3	2	2	2
35.	Filament	**P**ubescent **G**labrous	G	G	G	P	P	P	P	P	G	G	G	G	P	P	P	P	G	P	P	P	G	G	P	P	P
36.	Filament length	0.40-0.80 cm **(1)** 0.80-1.00 cm **(2)** > 1.00 cm **(3)**	2	2	1	2	2	1	1	1	2	2	1	1	3	2	3	3	3	2	2	1	3	3	2	2	1
37.	Filaments insertion	At base of corolla Above corolla base	Ab	Ab	Ab	Ab	Ab	Ab	Ab	Ab	Ab	Ab	Ab	Ab	At	At	At	At	At	At	At	At	At	At	At	At	At
38.	Style	**N**ot persistent **P**ersistent	P	P	P	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	P	P	N	N	N
39.	Style length	0.40-0.60 cm **(1)** 0.61-1.00 cm **(2)** 1.10-1.50 cm **(3)**	2	2	1	3	3	1	1	1	2	2	2	1	3	3	2	2	3	3	3	2	3	2	2	2	1
40.	Style texture	Glabrous Glandular	Gl	Gl	Gl	G	G	G	G	G	Gl	Gl	Gl	Gl	G	G	G	G	Gl	Gl	Gl	Gl	G	G	Gl	Gl	Gl
41.	Stigma	**2**-lobed **4**-lobed	2	2	2	2	2	4	2	2	2	2	2	2	2	2	2	2	4	2	2	2	4	4	4	4	4
42.	Stigma surface	**I**rregular **W**arty	W	W	W	W	W	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	W	W	I	I	I
43.	Ovary	Glabrous Glandular	Gl	Gl	Gl	G	G	G	G	G	G	G	G	G	G	G	G	G	Gl	Gl	Gl	Gl	G	G	Gl	Gl	Gl
44.	Ovary length	0.30-0.50 cm **(1)** 0.51-0.80 cm **(2)** >0.80 cm **(3)**	2	2	1	2	2	1	1	1	1	1	2	2	3	3	2	3	3	2	2	1	3	2	1	1	1
45.	Capsule shape	**O**void **E**llipsoid	E	E	E	E	E	E	E	E	E	E	E	E	O	O	O	O	O	O	O	O	O	O	O	O	O
46.	Capsule surface	**G**labrous **W**rinkled	G	G	G	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	G	G	G
47.	Seed colour	**Y**ellow **B**rown	Y	Y	Y	B	B	B	B	B	B	B	B	B	B	B	B	B	Y	B	B	B	Y	Y	B	B	B
48.	Seed shape	**O**val **E**lliptic	E	E	E	E	E	O	O	O	O	O	O	O	O	O	O	O	O	E	E	E	O	O	O	O	O
49.	Seed surface	**G**labrous **W**rinkled	G	G	G	W	W	W	W	W	G	G	G	G	W	W	W	W	G	G	G	G	G	G	G	G	G

Table 2. (Cont'd.).

No	Character	State	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
1.	Habit	Annual Perennial	P	P	P	A	A	A	A	A	A	A	A	A	P	P	P	P	P	P	P	P	P	P	P	P	P
2.	Parasitic on	Cultivated plants Wild plants	W	C	W	C	C	C	C	C	C	C	C	C	C	C	C	C	C	W	W	W	W	W	C	C	C
3.	Stem	Unbrached Branched	U	U	U	U	U	U	U	U	U	U	U	U	B	B	B	B	B	B	B	B	B	B	B	B	B
4.	Stem texture	Glabrous Tomentose Villous Glandular pubescent	G	G	G	P	P	T	T	T	T	T	T	T	T	T	T	T	Gl	T	T	T	P	P	V	V	V
5.	Stem cross section	Cylindrical Angular Irregular	I	I	I	C	C	C	C	C	C	C	C	C	C	C	C	C	C	A	A	A	I	I	A	A	A
6.	Leaf (Scales) duration	Leafless Leafy	L	L	L	Ls	Ls	Ls	Ls	Ls	Ls	Ls	Ls	Ls	L	L	L	L	Ls	Ls	Ls	Ls	L	L	Ls	Ls	Ls
7.	Leaf (scales) arrangement	Spirally Imbricate	S	S	S	-	-	-	-	-	-	-	-	-	S	S	S	S	-	-	-	-	I	I	-	-	-
8.	Leaf shape	Ovate Ovate-lanceolate Lanceolate	L	L	L	-	-	-	-	-	-	-	-	-	OL	OL	OL	OL	-	-	-	-	O	O	-	-	-
9.	Leaf texture	Tomentose Glandular	G	G	G	-	-	-	-	-	-	-	-	-	T	T	T	T	-	-	-	-	V	V	-	-	-
10.	Leaf apex	Obtuse Acute	A	A	A	-	-	-	-	-	-	-	-	-	O	O	O	O	-	-	-	-	O	O	-	-	-
11.	Pedicel hairs	Absent Scattered Dense	A	A	A	-	-	-	-	-	-	-	-	-	S	S	S	S	S	S	S	S	D	D	A	A	A
12.	Inflorescence type	Spike Raceme	S	S	S	S	S	S	S	S	S	S	S	S	R	R	R	R	R	R	R	R	R	R	R	R	R
13.	Inflorescence structure	Loose Dense	D	D	D	D	D	L	L	L	L	L	L	L	L	L	L	L	D	D	D	D	D	D	L	L	L
14.	Bract shape	Ovate Triangular Lanceolate	L	L	L	L	L	O	O	O	T	T	T	T	L	L	L	L	T	L	L	L	O	O	T	T	T
15.	Bract size	Shorter than calyx Equal Calyx	E	E	E	E	E	E	E	E	E	E	E	E	S	S	S	S	S	S	S	S	S	S	S	S	S
16.	Bract length	030-0.40 cm **(1)** > 0.40 cm **(2)**	2	2	1	2	2	2	2	2	2	2	2	2	2	2	2	2	1	1	1	1	1	1	1	1	1

Table 2. (Cont'd.).

No	Character	State	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
17.	Bracteoles	**Absent** **Two**	A	A	A	A	A	T	T	T	A	A	A	A	T	T	T	T	T	T	T	T	T	T	T	T	T
18.	Calyx shape	**Cupular** **Campanulate**	Ca	Ca	Ca	C	C	Ca	Ca	Ca	Ca	Ca	Ca	Ca	Ca	Ca	Ca	Ca	C	C	C	C	Ca	Ca	C	C	C
19.	Calyx length	0.40-0.80 cm **(1)** > 0.80 cm **(2)**	2	2	2	2	2	2	2	1	2	2	2	2	2	2	2	2	2	2	2	1	2	2	1	1	1
20.	Calyx apex	**2**-lobed **4**-lobed **5**-lobed **6**-lobed	2	2	2	2	2	5	5	5	5	5	5	5	**4**	**4**	4	4	2	4	**4**	4	6	6	5	5	5
21.	Calyx lobe shape	Lanceolate **Triangular**	T	T	T	T	T	T	T	T	T	T	T	T	L	L	L	L	L	L	L	L	L	L	L	L	L
22.	Calyx lobe tip	Entire **Toothed**	T	T	T	E	E	T	T	T	T	T	T	T	E	E	E	E	E	E	E	E	E	E	L	L	L
23.	Corolla colour	**White** **Yellow** **Blue** **Violet**	B	B	B	W	W	V	W	W	W	W	W	W	B	B	B	B	W	V	V	V	W	W	Y	Y	V
24.	Corolla length	0.80 –1.00 cm **(1)** 1.10-1.50 **cm (2)** > 1.50 cm **(3)**	3	3	2	2	2	3	2	1	3	3	2	2	3	3	2	2	3	3	3	2	3	3	2	2	2
25.	Corolla shape	**Campanulate** **Bilabiate**	B	B	B	C	C	B	B	B	C	C	C	C	B	B	B	B	B	C	C	C	B	B	B	B	B
26.	Corolla	**Straight** **Curved**	C	C	C	C	C	C	C	C	S	S	S	S	S	S	S	S	C	S	S	S	S	S	C	C	C
27.	Corolla upper lobe	Entire **2**-lobed	2	2	2	E	E	2	2	2	E	E	E	E	2	2	2	2	2	E	E	E	2	2	E	E	E
28.	Corolla lower lobe	Entire **3**-lobed	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	E	E	E	3	3	E	E	E
29.	Corolla lobes length	Equal Lower lobe shorter than upper Lower lobe longer than upper	Ll	Ll	Ll	E	E	Ls	Ls	Ls	E	E	E	E	Ll	Ll	Ll	Ll	Ls	E	E	E	Ls	Ls	E	E	E
30.	Corolla lobe tip	Entire **Toothed**	T	T	T	E	E	T	T	T	E	E	E	E	E	E	E	E	E	E	E	E	T	T	E	E	E
31.	Corolla upper part	Whitish to pale blue Dark blue to violet Brown	W	W	W	D	D	D	D	D	D	D	W	W	W	W	W	W	B	D	D	D	B	B	D	D	D
32.	Corolla dorsal line	**Geniculate** **Inflated**	G	G	G	G	G	G	G	G	G	G	G	G	I	I	I	I	I	I	I	I	I	I	I	I	I

Table 2. (Cont'd.).

No	Character	State	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
33.	Anther	**G**labrous **V**illous	G	G	G	V	V	V	V	V	G	G	G	G	V	V	V	V	G	V	V	V	G	G	V	V	V
34.	Anther length	0.20-0.30 cm (**1**) 0.31-0.40 cm (**2**) > 0.40 cm (**3**)	3	3	3	2	2	1	1	1	3	3	2	2	3	2	2	3	3	2	2	2	3	3	2	2	2
35.	Filament	**P**ubescent **G**labrous	G	G	G	P	P	P	P	P	G	G	G	G	P	P	P	P	G	P	P	P	G	G	P	P	P
36.	Filament length	0.40-0.80 cm (**1**) 0.80-1.00 cm (**2**) > 1.00 cm (**3**)	2	2	1	2	2	1	1	1	2	2	1	1	3	2	3	3	3	2	2	1	3	3	2	2	1
37.	Filaments insertion	At base of corolla Above corolla base	Ab	Ab	Ab	Ab	Ab	Ab	Ab	Ab	Ab	Ab	Ab	Ab	At	At	At	At	At	At	At	At	At	At	At	At	At
38.	Style	**N**ot persistent **P**ersistent	P	P	P	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	P	P	N	N	N
39.	Style length	0.40-0.60 cm (**1**) 0.61-1.00 cm (**2**) 1.10-1.50 cm (**3**)	2	2	1	3	3	1	1	1	2	2	2	1	3	3	2	2	3	3	3	2	3	2	2	2	1
40.	Style texture	Glabrous Glandular	Gl	Gl	Gl	G	G	G	G	G	Gl	Gl	Gl	Gl	G	G	G	G	Gl	Gl	Gl	Gl	G	G	Gl	Gl	Gl
41.	Stigma	**2**-lobed **4**-lobed	2	2	2	2	2	4	2	2	2	2	2	2	2	2	2	2	4	2	2	2	4	4	4	4	4
42.	Stigma surface	**I**rregular **W**arty	W	W	W	W	W	I	I	I	I	I	I	I	I	I	I	I	I	I	I	I	W	W	I	I	I
43.	Ovary	Glabrous Glandular	Gl	Gl	Gl	G	G	G	G	G	G	G	G	G	G	G	G	G	Gl	Gl	Gl	Gl	G	G	Gl	Gl	Gl
44.	Ovary length	0.30-0.50 cm (**1**) 0.51-0.80 cm (**2**) >0.80 cm (**3**)	2	2	1	2	2	1	1	1	1	1	2	2	3	3	2	3	3	2	2	1	3	2	1	1	1
45.	Capsule shape	**O**void **E**llipsoid	E	E	E	E	E	E	E	E	E	E	E	E	O	O	O	O	O	O	O	O	O	O	O	O	O
46.	Capsule surface	**G**labrous **W**rinkled	G	G	G	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	W	G	G	G
47.	Seed colour	**Y**ellow **B**rown	Y	Y	Y	B	B	B	B	B	B	B	B	B	B	B	B	B	Y	B	B	B	Y	Y	B	B	B
48.	Seed shape	**O**val **E**lliptic	E	E	E	E	E	O	O	O	O	O	O	O	O	O	O	O	O	E	E	E	O	O	O	O	O
49.	Seed surface	**G**labrous **W**rinkled	G	G	G	W	W	W	W	W	G	G	G	G	W	W	W	W	G	G	G	G	G	G	G	G	G

Table 3. The codes and nucleotide base sequences of the 10 random, 10-mer RAPD primers and the five ISSR primers that have been used for DNA fingerprinting for the examined taxa of *Orobanche*.

Serial	RAPD primers		ISSR primers	
	Primer code	Primer base sequence	Primer code	Primer base sequence
1.	OPA-08	5'CCAGTACTCC'3	$(CAA)_5$	5'CAA CAA CAA CAA CAA'3
2.	OPA-10	5'GTGATCGCAG'3	$(GACA)_4$	5'GAC AGA CAG ACA GAC A'3
3.	OPB-03	5'CATCCCCCTG'3	$(GATA)_4$	5' GAT AGA TAG ATA GAT A'3
4.	OPB-07	5'ACGGATCCTG'3	$(CA)_6RG$	5'CAC ACA CAC ACA RG'3
5.	OPB-10	5'GGTGATCAGG'3	$(CTC)_4RC$	5' CTC CTC CTC CTC RC'3
6.	OPE-12	5'AGACCCAGAG'3		
7.	OPE-17	5'CTACTGCCGT'3		
8.	OPF-16	5'AACCCGGGAA'3		
9.	OPJ-01	5'CCCGGCATAA'3		
10.	OPU-17	5'CCCAGCTGTG'3		

ISSR fingerprinting: The ISSR reactions were conducted using 5 specific primers; the codes and sequence of the used primers are shown in Table 3. The amplification of ISSR markers was performed according to Nagaoka & Ogihara (1997). The reaction mixture consisted of Hot Start Master Mix of 12.5 µl, primer (10 mM) 2.0 µl, template DNA (50 mg/µl) 1.0 µl, dd H_2O up to 25 µl. Amplification was carried out in a HVD thermocycler programmed as follows: 94ºC/4 min (1cycle); 94ºC/2 min, 45ºC/1 min, 72ºC/2 min (45 cycles); 72ºC/5 min (1 cycle) and 4ºC (infinitive). A marker of 1 kb of a total of 7 bands ranging from 250 to 1000 bp was used as DNA molecular size standard. The ISSR products were electro-phrased in 1.5% agarose gel in TAE buffer (0.04 M Tris-acetate buffer, pH= 8 at 100 volt for 60 min. Like the RAPD bands, ISSR bands were visualized on UV-trans-illuminator and photographed using gel documentation system (Gel Doc-BioRad, 2000). Each experiment was repeated twice and only stable products were scored.

Data analyses: The relationship among the examined taxa were estimated based on differences among them in both morphological traits as well as ISSR and RAPD finger printing separately and in combination. The morphological traits were given codes ranging between 0 and 3 depending on the variation in the average value for the measured traits. The RAPD and ISSR finger printing revealed as bands were scored as 1 and 0 for the presence or absence of the bands, respectively and the data was then used for the analyses. In order to construct trees elucidating the relationships among the examined taxa, the coded data were analyzed using the software NTSYS-pc 2.1 (Rohlf, 2000) based on the Neighbor-joining tree building method based on a distance matrix (Saitou & Nei, 1987). All analyses were performed with the version 2.02 of the software package NTSYS-pc (Rohlf, 2000).

Results and Discussion

Relationship among taxa based on morphological variation: The relationship among the studied species of the genus *Orobanche* from Egypt and Saudi Arabia, based on morphological variation, was expressed as NJ and is shown in Figure 1. In this tree the 25 samples were clearly divided into two groups at a NJ distance coefficient of about 5.70; the first group included the samples of *O. purpurea*, *O. lavandulacea*, *O. ramosa*, *O. mutelii* and *O. aegyptiaca* and the second group was composed of samples representing *O. cernua, O. crenata, O. minor* and *O. pubescens*.

In the former group which comprised of 5 species of section *Trionychon,* the two samples of *O. purpurea* were delimited at a distance coefficient of about 5.50 from the other three species of the same section. The remaining taxa of the section *Trionychon* were distinguished into two clusters at a NJ distance coefficient of about 5.20. In the first cluster including the four samples of *O. aegyptiaca*, the two samples collected from Egypt were separated from the other two samples collected from Saudi Arabia at low distance coefficient of about 2.50. In the second cluster, *O. lavandulacea* is distinguished from the remaining two species (*O. ramosa and O. mutelii*) at distance coefficient of 5.20. On the other hand the three samples of *O. ramosa* were separated from the three samples of *O. mutelii* at distance coefficient of about 4.60.

In the second group that comprised of the 4 species of section *Orobanche*, the three samples of *O. cernua* were delimited from the remaining species of the same section at distance coefficient of about 5.00. At distance 4.80 the two samples of *O. crenata* were separated from the samples of the other two species of section *Orobanche*; *O. minor* and *O. pubescens*, these 2 species were separated at distance coefficient of about 3.20. Regarding the 4 samples of *O. pubescens*, the samples 1&2 collected from Egypt were clearly distinguished from the samples 3&4 collected from Saudi Arabia at a low distance coefficient of about 2.60. Also, the samples of *O. minor* collected from Egypt were separated from the other two samples (2&3) that collected from Saudi Arabia at a low distance coefficient of about 2.40.

The relationship among the examined taxa as revealed by analysis of morphological traits agrees with previous taxonomic studies on *Orobanche* established by Beck-Mannagetta (1930), Andary (1994) and Plaza *et al*. (2004). The two sections (*Orobanche* and *Trionychon*) were mainly identified by the structure of the calyx, corolla colors and the presence or absence of bracteoles. This result agrees with Pujadas-Salvà (2002) and Plaza *et al*. (2004). In addition to traditional taxonomy, Plaza *et al*. (2004) studied the micro-morphology of *Orobanche* seeds in the Iberian Peninsula and found enough variability to develop a key identifying *Orobanche* species according to the thickness of the epidermal anticlinal walls.

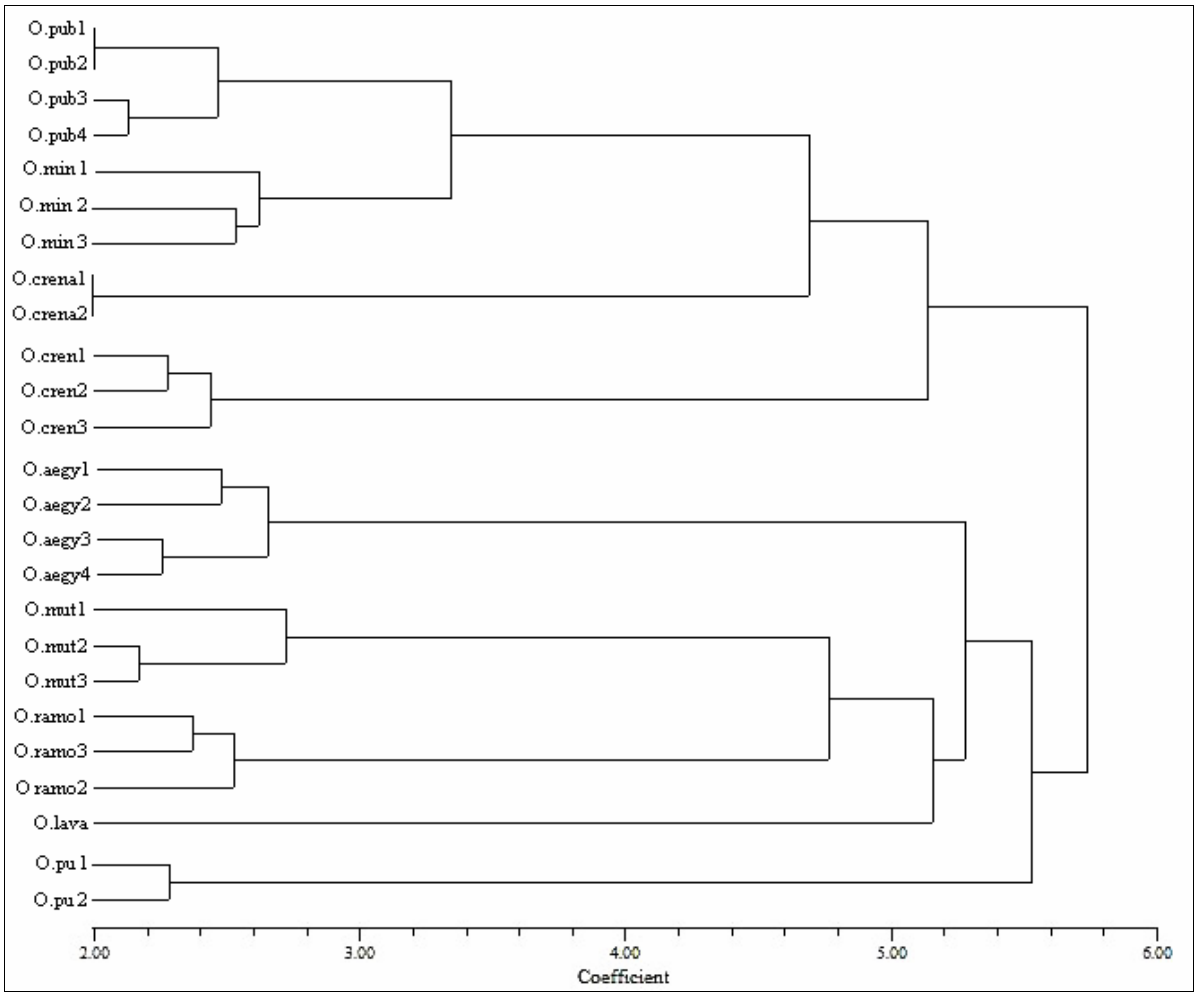

Fig. 1. NJ tree illustrating the relationships among the studied 25 taxa of *Orobanche* based on the analysis of morphological variation.

Within section *Trionychon*, *O. purpurea* was separated from the remaining species by its pubescent stem, imbricate leaves, ovate bracts, toothed corolla tip, persistent style and warty stigma surface. *O. aegyptiaca, O. lavandulaceae, O. mutelli* and *O. ramosa* share some similarities such as host ranges and constituted a species cluster within the section *Trionychon*. *O. lavandulaceae* was separated from the other species by its glabrous leafless stem, and two lobed calyx apex. *O. aegyptiaca* was separated from *O. mutelli* and *O. ramos*a by its relatively more branched stem, spirally arranged tomentose leaves, moderately dense to lax inflorescence, blue corolla, pubescent stamens and wrinkled seed surface. *O. mutelii* was treated by some authors as infraspecific variant of *O. ramosa* (Mohamed & Musselman, 2008). *O. ramosa* had a smaller calyx and corolla distinguishing it from *O. mutelii* with tomentose stem, hairy pedicle, large calyx and corolla, entire calyx tip, campanulate corolla, wrinkled capsule surface and elliptic seeds.

Within section *Orobanche*, the dendrogram showed that *O. crenua* was relatively isolated from the other members of the section analyzed (*O. crenata, O. minor* and *O. pubescens). O. crenua* is parasitic on many hosts including wild plants, differs from the rest in presence of leaves, blue corolla, glabrous capsule surface and yellow seeds. Within the other three species, *O. pubescens* was closely related to *O. minor* than to *O. crenata. O. pubescens* had a unique morphology and easily separated from the other species. It has pubescent stem, dense inflorescence, fragrant flowers, entire calyx tip and a long corolla which is usually white with purple veins. Although the 4 taxa of *O. pubescens* were quite close in the dendrogram, a slight divergence was found between the members collected in Egypt and those in Saudi Arabia.

However, it was found that, within the species, the geographical distribution effects on some morphological characters of the taxa under investigation. This was evident in the four *O. pubescens* samples and the three samples of *O. minor*. In the former species, the two samples from Egypt differ from the 2 samples from Saudi Arabia, in the corolla color and length, anther, filament and ovary lengths . In the later species (O. minor), the sample from Egypt differ from the two samples from Saudi Arabia, in the corolla color and the number of the stigma lobs. On the other hand, it was found that the host had no effect either on the morphological characters or in the relationships between the samples of the same species and our result disagreed with Román *et al*., (2003).

Table 4. Number and type of amplified bands generated by the ten used RAPD primers in the 25 taxa of *Orobanche*.

Band type	RAPD Primers and number of bands										
	OPA-08	OPA-10	OPB-03	OPB-07	OPB-10	OPE-12	OPE-17	OPF-16	OPJ-01	OPU-17	Total
Monomorphic	4	3	2	5	3	2	2	2	1	1	25
Unique	4	1	1	0	1	1	0	0	0	1	9
Polymorphic	11	8	4	13	12	4	4	3	3	7	69
Total bands	21	12	7	18	16	7	6	5	4	9	103
% Polymorphism	**71.4**	**75**	**71.4**	**72.2**	**81.3**	**71.4**	**66.7**	**60**	**75**	**77.8**	**75.7**

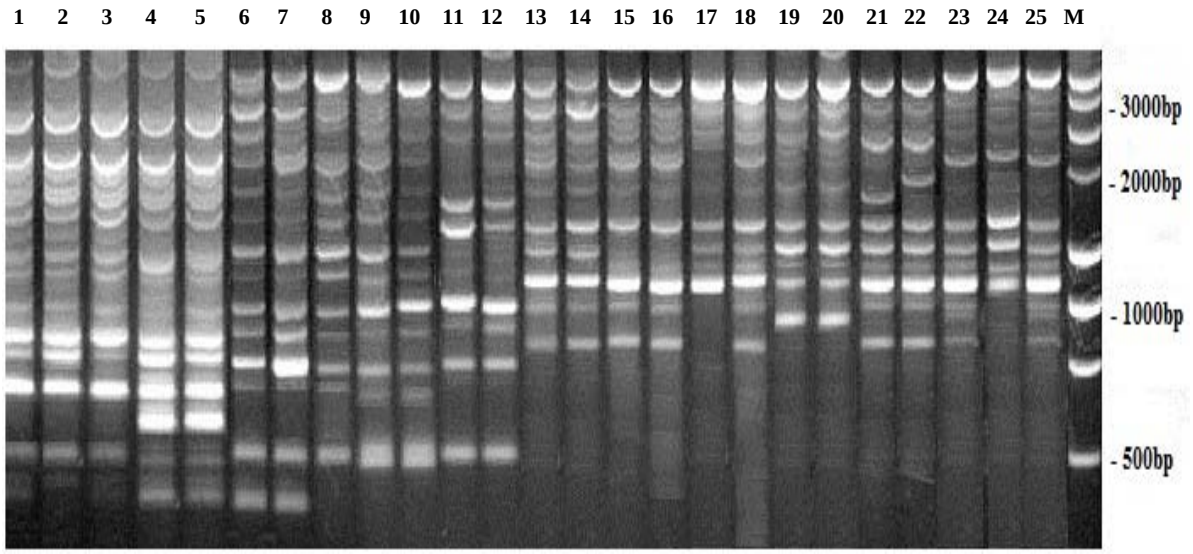

Fig. 2. Photograph illustrating RAPD finger printing of the studied 25 samples of *Orobanche* as revealed by the primer OPB-08. The lane to the right is a molecular size marker. Numbers on lanes 1-25 correspond to the serial numbers of samples as numbered in table 1.

Relationship among taxa based on RAPD and ISSR polymorphism: A total of 103 RAPD bands were revealed in the profiles by the used primers in 25 samples of *Orobanche*; of these 69 bands were polymorphic and 25 were monomorphic (Table 4). The polymorphic bands included nine unique bands that were revealed by six primers. The highest number of bands was produced by the primer OPA-08 (Fig. 2), the primer OPB-07 produced the highest number of polymorphic bands (13) and the primer OPB-7 produced the highest number of monomorphic and unique bands. The smallest number of bands (4 & 5 bands) was produced by the two primers OPJ-01 and OPF-16 respectively; the number of polymorphic bands was three for the primers OPJ-01 and OPF-16 with 60% and 75% polymorphism respectively. The percentage of polymorphism as revealed by the used primers ranged between 60% by the primer OPF-16 and 81.3% by the primer OPB-10, compared to the total polymorphism of 75.7% as shown in Table 4.

The number and molecular size range (in bp) of RAPD amplified bands produced in the 25 taxa of *Orobanche* by the ten primers are shown in Table 5. The three primers OPB-07, OPB-10 and OPA-08 generated more total products in all taxa compared to other primers. The products size ranges between 350 and 4000 bp. The primer OPB-07 produced different number of bands in the examined taxa, these numbers ranged from eight in *O. lavandulacea*, *O. mutelii* and *O. ramosa* to 18 in *O. cernua*. The primer OPA-10 produced bands ranging between 10 in *O. purpurea* and *O. ramosa* to 16 in *O. cernua*. The number of bands produced by the primer OPA-07 was also much higher than other primers; it ranged between seven in *O. cernua* to 17 in *O. aegyptiaca*. The other primers produced lower number of bands that appeared in a narrower size range; this is particularly true for the primers OPE-12, OPF-16 and OPJ-01 (Table 5).

The number of bands produced by the five ISSR primers in the 25 samples of *Orobanche* was much lower than those produced by the RAPD primers. The 5 primers tested produced a total of 76 bands including only 41 polymorphic bands (Fig. 3). The number of bands ranged between 16 revealed by the primer $(CA)_6RG$ and seven products as revealed by the primer $(CAA)_5$. The number and types of bands produced by all primers are given in Table 6. The primer $(GACA)_4$ produced a band that was present only in the sample of *O. lavandulacea* (lane 17) and also the primer $(CA)_6RG$ produced a unique band in sample 1 of *O. pubescens* that was collected from Egypt (lane 9). On the other hand, it was apparent that the ISSR profiling clearly differentiated between the *Orobanche* species belonging to section *Orobanche* from species belonging to section *Trionychon* as shown in Table 6.

Table 5. Number and molecular size range (in bp) of amplified bands produced in 25 *Orobanche* taxa by the used ten RAPD primers.

Ser	Taxon	RAPD Primers, number and size range of bands.										
		OPA-08 (350-4000)	OPA-10 (500-4000)	OPB-03 (500-3000)	OPB-07 (500-3000)	OPB-10 (500-3000)	OPE-12 (1000-3000)	OPE-17 (1000-3000)	OPF-16 (500-2000)	OPJ-01 (1000-2000)	OPU-17 (500-3000)	Total Bands
1.	*O. cernua* 1	8	11	6	18	15	6	5	3	3	7	82
2.	*O. cernua* 2	7	11	6	18	16	6	5	3	3	7	79
3.	*O. cernua* 3	7	10	6	17	15	7	5	3	3	7	80
4.	*O. crenata* 1	14	8	7	15	14	4	4	3	3	7	73
5.	*O. crenata* 2	13	8	6	14	14	4	4	3	3	7	76
6.	*O. minor* 1	11	9	6	13	14	4	4	3	4	7	75
7.	*O. minor* 2	11	9	5	13	14	4	4	3	4	7	74
8.	*O. minor* 3	14	8	6	13	14	4	4	3	4	7	77
9.	*O. pubescens* 1	10	9	6	12	14	4	4	3	3	8	73
10.	*O. pubescens* 2	10	9	6	12	14	4	4	3	3	8	73
11.	*O. pubescens* 3	9	9	6	11	13	3	4	3	3	8	69
12.	*O. pubescens* 4	9	8	6	11	12	3	4	3	3	8	67
13.	*O. aegyptiaca* 1	17	8	4	10	11	4	4	3	3	7	71
14.	*O. aegyptiaca* 2	16	8	4	10	11	4	4	3	3	7	70
15.	*O. aegyptiaca* 3	15	6	3	9	11	4	4	3	3	7	65
16.	*O. aegyptiaca* 4	15	7	3	9	11	4	4	3	3	7	66
17.	*O. lavandulacea*	7	6	4	8	12	4	4	4	3	7	55
18.	*O. mutelii* 1	14	8	4	8	11	4	4	3	3	7	66
19.	*O. mutelii* 2	12	7	4	9	11	4	4	3	3	7	64
20.	*O. mutelii* 3	12	7	3	8	11	4	4	3	3	7	62
21.	*O. purpurea* 1	8	4	3	9	10	4	5	4	3	7	54
22.	*O. purpurea* 2	8	4	3	9	10	4	5	4	3	7	54
23.	*O. ramose* 1	16	6	4	9	11	4	4	3	3	7	67
24.	*O. ramose*. 2	15	6	4	8	11	4	4	3	3	7	65
25.	*O. ramose* 3	16	6	4	8	10	4	4	3	3	7	65

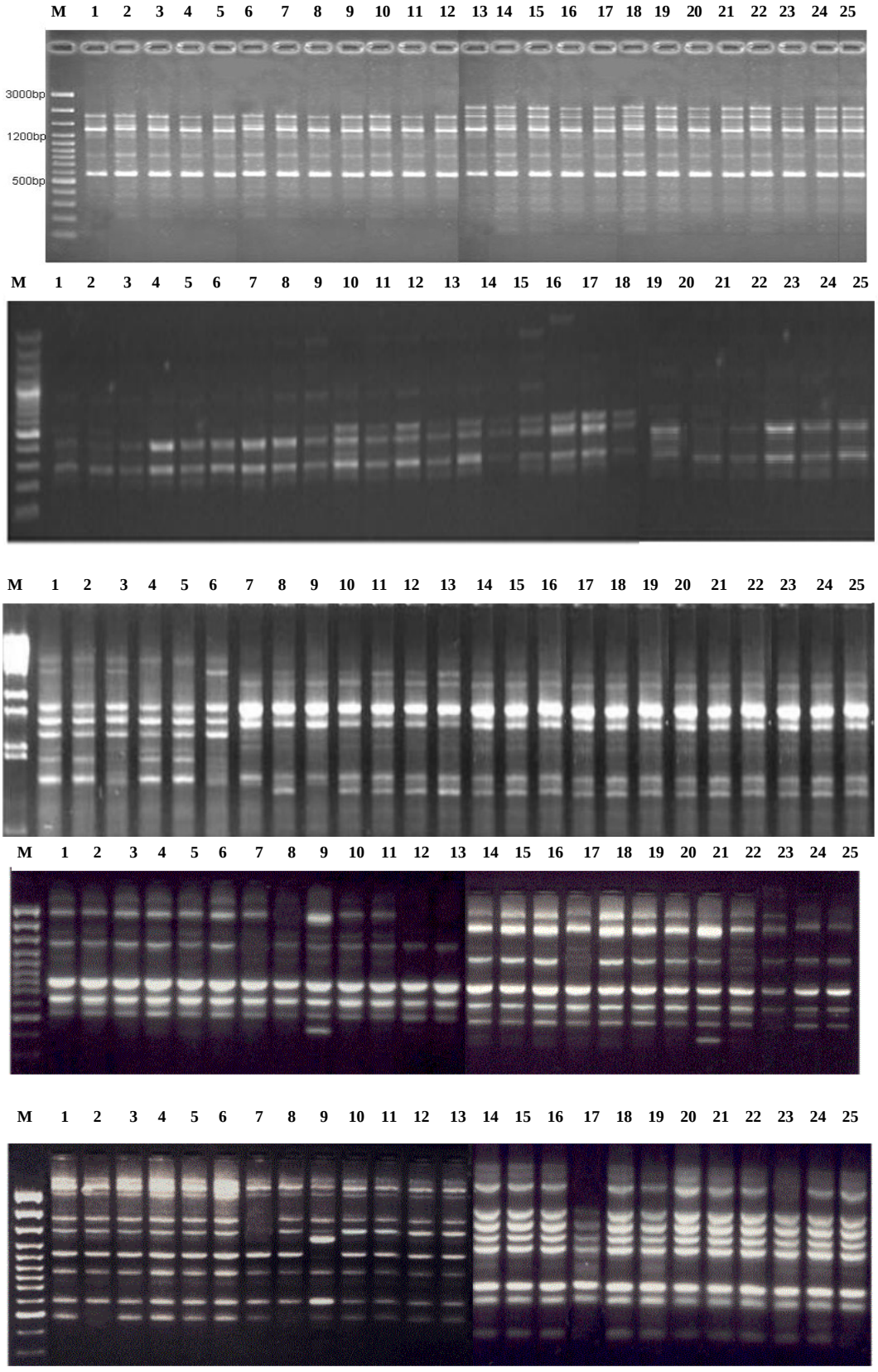

Fig. 3. Photographs illustrating example of the ISSR finger printing for the studied 25 samples of *Orobanche* as revealed by five ISSR primers; primer codes are as follows: A=Primer 1, B=Primer 2, C=Primer 3, D=Primer 4, E=Primer 5 (see table 3). Number on lanes 1-25 correspond to the serial numbers of *Orobanche* samples as numbered in table 1.

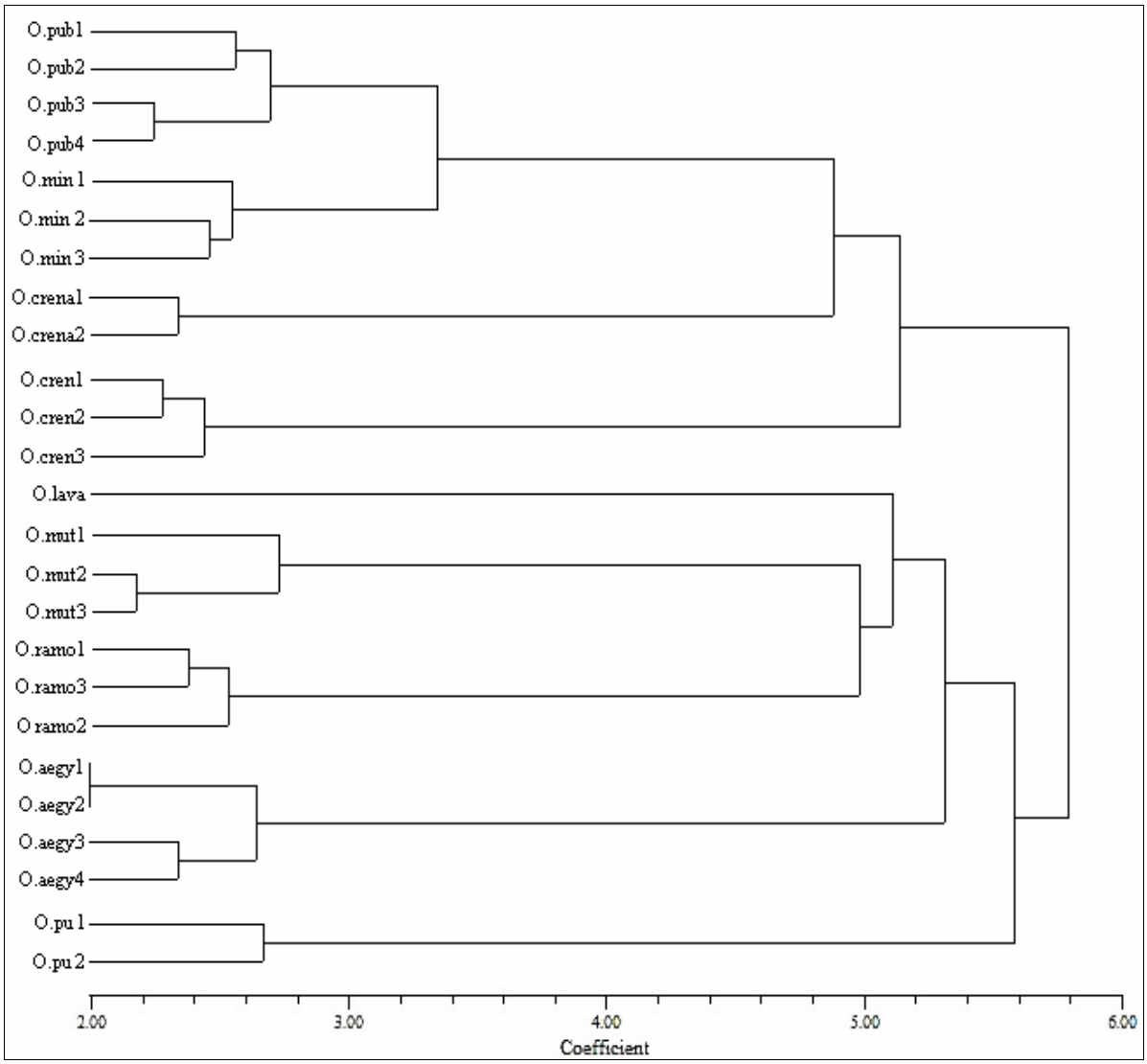

Fig. 4. NJ tree illustrating the relationships among the studied 25 taxa of *Orobanche* based on the analysis of RAPD and ISSR molecular fingerprinting.

Table 6. Number and type of amplified bands generated by the five used primers in 25 taxa of *Orobanche*.

Band type	ISSR primers and number of bands					Total
	$(CAA)_5$	$(GACA)_4$	$(GATA)_4$	$(CA)_6RG$	$(CTC)_4RC$	
Monomorphic	4	2	2	3	2	13
Unique	0	1	0	1	0	2
Polymorphic	3	8	10	11	9	41
Total bands	7	11	12	16	11	76
% Polymorphism	**42.9**	**81.8**	**83.3**	**75**	**81.8**	**56.6**

The tree produced by the analysis of RAPD and ISSR markers profiles using Neighbor Joining (NJ) method is shown in Figure 4. The topology of this tree generally resembles that of the tree based on the morphological variation (Fig. 1).The studied taxa were separated in two groups at NJ distance coefficient of about 5.80, one comprised of the 5 species of section *Trionychon* and the second comprised of the four species of section *Orobanche*. In the former group the two samples of *O. purpurea* were clearly separated at a distance of about 5.60 on the NJ distance scale from the remaining species of section *Trionychon*. The remaining four species (*O. lavandulacea*, *O. mutelii*, *O. ramosa*, *O. aegyptiaca*) were delimited into two clusters at a distance coefficient of about 5.20. The first cluster comprised of the four samples of *O. aegyptiaca*, where the samples 1&2 that were collected from Egypt were distinguished from the two samples 3&4 that were collected from Saudi Arabia at low distance coefficient of about 2.60.

In the second cluster, *O. lavandulacea* is separated from the other two species (*O. mutelii*, *O. ramosa*) at distance coefficient of about 5.00. At NJ distance coefficient of about 4.90 the three samples of *O. ramosa* were separated from the other three samples of *O. mutelii*. In the second group of the NJ tree based on RAPD and ISSR polymorphism, the four studied species of section *Orobanche* were delimited into two clusters at distance coefficient of about 5.10. The first cluster comprised of the three samples of *O. cernua* that are separated from the other three species (*O. crenata*, *O. minor*, *O. pubescens*). In the second cluster at distance coefficient of about 5.00, the two samples of *O. crenata* were distinguished from the other two species (*O. minor*, *O. pubescens*). The latter two species were delimited from each other at distance coefficient of about 3.40. Regarding the three samples of *O. minor*, sample 1 that was collected from Egypt was distinguished from the samples 2&3 that were collected from Saudi Arabia at low distance coefficient of about 2.50. At low distance coefficient of about 2.60 the two samples (1&2) of *O. pubescens* that were collected from Egypt were separated from samples (3&4) that were collected from Saudi Arabia.

In this study, the NJ trees based on morphological and molecular data (Figs. 1&4) produced similar taxonomic results that splitting the examined *Orobanche* species into two main groups; the first group contained the species related to section *Orobanche* and the second one contained the species related to section *Trionychon*. This result, largely in agreements with previous taxonomic studies based on chemotaxonomy of *Orobanche* (Velasco *et al*. (2000)), and chemo- taxonomical and molecular studies conducted by Román *et al*. (2003) and Manen *et al*. (2004). According to Holub (1977, 1990), Teryokhim *et al*. (1993) and Gevezova *et al*. (2012), the two studied sections were recognized as separated genera: *Orobanche* and *Phelipanche* (syn. *O.* sect.*Trionychone*). Moreover, Pujadas-Salvà (2007) placed *Phelipanche* as subgenus of *Orobanche*.

According to Stoyanov (2009), subsect. *Minores* was represented by six species in Bulgarian flora; and because of the high morphological similarity of five of them, including *O. minor* and *O. pubescence*, they were grouped in aggregate *O. minor* (Chater and Webb, 1972; Gilli, 1982; Delipavlov, 1995). According to Musselman (1986, 1994) this aggregate consisted of one species with wide morphological variability caused by the host plant and was poorly resolved even by broad- scale molecular phylogenetic analysis (Manen *et al*., 2004; Schneeweiss, 2007; Park *et al*., 2008).

According to Sharawy (2008) O. *minor* was more related to *O. crenata* than *O. pubescence* depending on variation in polymorphism in seed protein components. The analysis of the combined data of ISSR and RAPD as well as the morphological characters indicated that *O. minor* was closer to *O. pubscence* than *O. crenata*. In section *Trionychon, O. ramosa* and *O. mutelli* were closely related in the analysis based on morphological and molecular characters, the grouping of these two species together is in agreement with the view of Sharawy (2008).

In conclusion, the relationships between the studied species of *Orobanche*, based on variation in morphological and molecular characters, agree with their previous delimitation in two genera: *Orobanche* with four species and *Phelipanche* (syn. *O.* sect. *Trionychon*) with five species (Holub, 1977, 1990; Teryokhim *et al*., 1993; Gevezova *et al*., 2012). The case of high similarity that was detected between *O. pubescens* and *O. minor*, disagree with Musselman (1986, 1994) and Stoyanov (2009). This study confirmed the relationship between *O. ramosa* and *O. mutelli*, the later was treated by some authors as infraspecific variants of *O. ramosa* (Mohamed & Musselman, 2008). The result demonstrated clustering not only by species but also, in most cases, by geographically isolated population (Gevezova *et al*., 2012).

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