MOLECULAR AND MORPHOLOGICAL STUDIES OF DODDER '*CUSCUTA SPP.*' IN EGYPT

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(Manuscript received 17 September 2005)

Abstract

Dodder *"Cuscuta sp."* is leafless twining parasite with small flowers in heads or clusters. It has different higher plant hosts except *C. epilinum*, which grows only on flax. It has no normal roots or leaves. It produced haustoria's to attach its host. Morphological identification of *Cuscuta spp* in Egypt probably is not normally easy, so molecular studies on protein and DNA levels were used as genetic markers for the studied dodder population identification in Egypt during the period of 1998 to 2002.

Electrophoretic patterns of total seed protein as revealed by polyacrylamide gel electrophoresis (SDS-PAGE) and DNA-RAPD amplified in dodder fresh stems samples by arbitrary 12 primers were used as genetic markers of *Cuscuta sp.* Results of protein and DNA analysis showed that the dodder (Family Cuscutaceae) has polymorphic characters on molecular level and concluded that the dodder family has two genera *Grammica (G. campestris)* and *Cuscuta (C. pedicellata, C. planiflora and C. epilinum)* species.

INTRODUCTION

Populations of a species, which are reproductively isolated, for instant by distance, may diverge from each other through drift and/or differential selection. If they have diverged sufficiently they may be called two ecotypes, forms or even subspecies, (Wolff & Morgan 1998). It is generally accepted that morphological characters and ecological niche are good guidelines to distinguish two forms or subspecies within a species (Molgaard, 1976). Differences in a number of morphological characters, especially of the reproductive organs of plants, often provide the most conspicuous means of distinguishing closely related species, or are used in studying the differentiation level among conspecific populations.

Taxonomists tried to distinguish the morphological diversity of taxa especially using the characters of leaves and floral parts (Rajaseger *et al.,* 1997). Morphological

characters of some species described in the botanical keys fail to reveal any large scale of differentiation (Ball, 1968). Alternative approaches for intra– and inter–specific classification have been reported based on geographic origin, morphology, karyotype or isozyme analysis (kaser and Steiner, 1983). These criteria are either influenced by environmental factors and stage of plant development or reveal only limited variation.

Information on genetic diversity and relationships within and among species is essential for the efficient utilization of plant genetic–resource collections. In turn, these results should be supported by cytological and biochemical studies (Chooi, 1971 and Pier Giovanni *et al.*, 1995). Recently molecular techniques have been used to study the extent of differentiation among populations, ecotypes forms and subspecies. Different molecular markers show different levels of genetic divergence, depending amongst other criteria on the rate of evolution of the specific marker.

The study of differentiation among closely related taxonomic units for a range of characters, from morphological characters, protein to repetitive DNA, not only sheds light on the classification of the taxonomic units under study, but also teach us about the evolution of characters and molecules.

The advent of molecular markers, e.g. random amplified polymorphic DNA (RAPD) and restriction fragment length Polymorphism (RFLP), has provided a different approach to study genetic diversity. These markers have been used to classify plants that cannot be identified by morphological characters. In addition, RAPD markers have the potential to reveal large amounts of variation with good coverage of the entire genome (Melchinger *et al.*, 1994).

Dodder taxonomy is difficult for several reasons: 1. There is no inherent morphological variability with the dodder populations. 2. It have no leaves, it produce short abnormal roots, which separate from soil immediately after their shoot emergence and attachment to the host and 3. Environmental factors may influence the morphology of the dodder plants. So, molecular studies on protein and DNA polymorphism can be useful tools as genetic markers to overcome this difficulty. Electrophoresis is the most simple and quite powerful tool for separation and identification of DNA and gene products (Pauls, 1996 and EL-Nady, 2000).

Cuscuta is a leafless twining parasite with small flowers in heads or clusters. Flowers sessile or short pedicelled. Calyx 4-5 cleft. Corolla lobed 4-5 united at base into tubes. Stamens 4-5 inserted at the throut, usually with a whorl of granged scales

opposite to end below the stamen. Styles 2 free or united with capitates or linear stigma, fruit capsule. Family Cuscutaceae is represented by three genera; *Monogyna, Cuscuta* and *Gramica* (Chrteck & Osbronva, 1991).

Few studies have focused on the genetic polymorphism among species of the genus *Cuscuta,* while great morphological characters were detected among them (Täckholm, 1974).

In Egypt, eight species of *Cuscuta* were reported by Bolous (2000); these are: *C. monogyna, C. chinesis, C. campestris, C. pedicellata, C. palaestina, C. epilinum, C. planiflora* and *C. approximate.* Recently, El-Anany (2003) recorded four species of *Cuscuta* (*C. padicellata, C. planiflora, C. epilinum* and *C. campestris*).

Therefore, the aim of the present study is the differentiation between 4 species of this genus namely, *Cuscuta padicellata, C. planiflora, C. epilinum* and *C. campestris,* and to find the degree of relationships among them by molecular analysis.

MATERIALS AND METHODS

Morphological and molecular analysis to differentiate between *Cuscuta spp* in Egypt.

1- *Cuscuta spp.* samples collection:

Cuscuta pedicellata was collected in 1998/1999/2000 from Sanoris village, Fayoum governorate where it was growing on *clover "Trifolium alexandrnum"*; *C. planiflora* from Behera governorate where it was growing on *Urtica sp.*; *C. epilinum* collected 2000/01 from Kalube, Kalubia governorate where it was growing on flax '*Linum usitatissmum'* and G. *campestris* was collected in 1998-2002 from fields of clover in Giza governorate.

2-Protein-polyacrylamide gel electrophoresis (SDS-PAGE):

The extracted protein from decoated ground seeds samples (10 g each) was suspended in 100 ml of extraction buffer, left for 1 h at 4°C, clarified by centrifugation at 4°C (1000 rpm), fractionated by SDS-PAGE and detected according to the method of Laemmlia (1970) as modified by Studier (1973).

3- DNA-electrophoresis (RAPD-PCR):

RAPD – PCR was carried out according to the procedure given by Bagheri *et al.* (1995) with minor modification.

a. Isolation of plant genomic DNA:

Isolation of DNA from 10 g stems in case of each sample was carried out using cetyltrimethyl ammonium bromide CTAB method (Giannino *et al.*, 1989).

PCR Reactions: Amplification reaction was carried out in a total volume of 50 µl as described by Bagheri *et al.* (1995). Each reaction mixture contained 50 ng genomic DNA as a template, 0.4 M decamer oligonucleotide primer (Operon Technologies, Alameda, CA) kit B, 12 primers as shown in Table (1), Two units of Taq DNA polyrnerase (Promega Corp., Madison, WI, USA), 5 µl of 10x buffer [500 mM KC1, 100mM Tris-HC1 (pH 9.0) and 1% Triton-100], 1.5 mM M $MgCl_2$, 0.2 mM dNTPs (dATP, dCTP, dTTP, dGTP) and deionized H_20 up to 50 µl. The reaction was overlaid with a drop of mineral oil to avoid evaporation. PCR amplification was performed in a perkin-elmer thermal cycler 480 (Norwalk, CT) for 40 cycles after initial denaturation for 3 min at 94°C. Each cycle consisted of denaturation at 94°C for 1 min, annealing at 36°C for 1 min, extension at 72°C for 2 min. The primer extension segment was extended to 5 min at 72°C in the final cycle. The amplification products were resolved by electrophoresis in a 1% agarose gel at 60 volts for 3.5 h with 1X TBE (Tris-borate EDTA) buffer. Standard DNA (X DNA IBstEII digest, with bands of 8.454, 7.242, 6.369, 5.686, 4.822, 4.324. 6.675, 2.323. 1.929, 1.371, 1.264, 0.702, 0.224 and 0.117 Kb was loaded as well.

Table (1): Decamer oligonucleotide primers used in PCR-RAPD techniques.

Primer	Oligonuclutide
OPA – 01	CAG GCC CTT C
OPB – 07	GCT GAC GCAG
OPB – 09	TGG GGG ACTC
OPB – 11	GTA GAC CCGT
OPB – 18	CCA CAG CAGT
OPB – 19	ACC CCC GAAG
OPB – 20	GGA CCC TTAC
OPD – 10	GGT CTA CACC
OPE – 19	ACG GCG TATC
OPZ – 01	TCT GTG CCAC
OPZ – 03	CAG CAC CGC A
OPZ – 08	GGG TGG GTA A

b- Photography of agarose gels

Agarose gels were stained by ethidium bromide and visualized on UV trans illuminator filter by ultraviolet light (302 nm wavelength) to detect the ethidium bromide/DNA complex. Photography was made by using Polaroid film type 57 (ASA

3000) (Sambrook *et al.*, 1989).

RESULTS AND DISCUSSION

Morphological characters of *Cuscuta* species:

One of the most diagnostic characters is the infra-stamen scale, which connected to the base of corolla (Fig 1). In *Cuscuta pedicellata*, the scales are simple, or bifid, short appendages, number of appendages is few (3-10), with 0.75-0.95 mm long. Scales reaching the stamen, the apex dentate, ovate-oblong and fimbriation margin. *C. planiflora*, the scales is bifid or simple, few appendages (3-10), with 0.7-0.9 mm long. Scales reaching stamens, oblong-ovate, the margin short fimbriation around obtuse apex. In *C. epilinum*, scales are bifid, small, umbel like, shorter than tube, fimbriation, number of appendages (10-17), with short appendage 0.1- 0.3 mm long. Scales extending beyond sinues of the corolla. G. *campestris*, bifid scales with long appendages (0.9- 1.2 mm long), with 11-25 appendages, scales reaching stamens, oblong-ovate and entire margin.

Gynecium is another important characters (Fig 2), in *C. pedicellata*, style is short (1.1-1.4 mm) long, styles reduced of two small tubercles, each ending in a subulate stigma. Stigma linear, 0.5mm long, subsessile, thin and elongate. Style and stigma as long or shorter than the ovary. Ovary globose, conical, slightly depressed at the apex. *C. planiflora*, style and stigma as long or longer than ovary, 1.2- 1.75 mm in long, stigma is linear, ovary globose. *C. epilinum*, style and stigma much shorter than ovary, and the style very short, stigma 0.5 mm, elongate, ovary globose, slightly depressed at the apex, longer than style and stigma. In *G. campestris*, style and stigma $\pm$ as long as ovary, styles 2, 1-1.2 mm long, stigma capitate, ovary 1-1.2 mm globose.

In the present work, a comparative analysis of relative concentration and mobility rate of different protein bands detected in the studied of different *Cuscuta* species using SDS–PAGE technique are presented in Figure (3) and Table (2).

In spite of thirty three protein bands at different molecular weights for the SDS-PAGE analysis, ten major bands were shown in case of all species studied at the following molecular weights: 72.628, 59.416, 55.0, 46.774, 40.250, 34.87, 30.398, 28.662, 18.255 and 14.638 K.Da. In addition, five characteristic protein bands in case of *C. epilinum* numbers: 2, 8, 10, 17 and 27 of molecular weights: 86.078, 58.055, 56.726,

39.739 and 27.025 K.Da., respectively; three characteristic protein bands in case of *G. campestris* numbers: 1, 9 and 26 of the molecular weights: 90.161, 72.628 and 57.608 K.Da., respectively; two characteristic protein bands in case of *C. pedidellata* numbers: 4 and 30 of the molecular weights: 76.073 and 19.65 K.Da.and two characteristic protein bands in case of *C. planiflora* numbers: 3 and 24 at the molecular weights: 77.257 and 29.614 K.Da., respectively. All the previous patterns are discriminating for each taxa.

The presence or absence of protein bands can be used as a diagnostic character for a group or a certain taxon (Jensen and Lixue, 1991). Abou El-Enain (1995), considered banding profiles as traits to study genetic variation among plant taxa of Solanaceae. Driedger *et al.* (1994), Hussain *et al.* (1986), Rao *et al.* (1990), Badr (1995), Badr *et al.* (1998), Ladizinsky and Hymowitz (1979), El- Shanshouri (1997), Ghareeb *et al.* (1999), EL-Naggar and Soliman (1999) and El-Nady (2000) reported that the differences in electrophoretic pattern of seed proteins were primarily the consequence of genotypic rather than of environmental influences. They also considered protein markers are more rapid and reliable tool to discriminate taxa than by comparison of morphological and physiological characteristics. They also reported that electrophoretic analysis of non–germinating seeds is useful for resolving questions of seed identification and for screening seeds of unknown origin. Many authors also used SDS-PAGE technique to identify different cultivars and species within many families and genera, Sammour (1992).

DNA (RAPD) analysis

Random Amplified Polymorphic DNA (RAPD) analysis has been used in the present investigation to differentiate between the four species under study. Figure 4 and Table 3 showed the results of DNA analysis for the three different studied *Cuscuta* species and *Grammica campestris* where 171 RAPD bands resulted by applying twelve primers. Thirty-one major bands were shared by all studied species.

Meanwhile, one common major band appeared in case of using primers: OPB–9, OPB-11 and OPZ-1. Two bands appeared in case of using primers: OPA-1, OPB–7, OPZ–3 and OPZ–8. Three major bands appeared in case of using primer OPD-10. Four major bands appeared in case of using primers: OPE–19, OPB–18 and OPB–19. Five major bands appeared in case of using primer OPB–20. In addition, primer OPB-7 show a discriminating band number 16 of 200 bp in case of *C. epilinum*, band 4 of 1300 bp in case of *G. campestris* and number 6 of 1100 bp for *C. pedicellata,* By using

primer OPB–9 band number 1 of 3269 bp is a characteristic band in case of *C. epilinum* and band number 11 of 600 bp in case of *G. campestris.* On the other hand, using primer OPB–11, four characteristic bands in case of G.*campestris* numbers: 6, 9, 10 and 11 of 1200, 900, 800 and 600 bp, respectively. Using primer OPB–18-band number 10 of 838 bp characterize *C. epilinum* and band number 16 of 300 bp characterize *C. pedicellata.* Using primer OPB–19 bands numbers: 7, 11, 14, and 18 of 1500, 1000, 750 and 400 bp, respectively characterize *C. epilinum* and three characteristic bands discriminate *G. campestris* (numbers 5, 8, and 15 at 1800, 1400 and 700). Using primer OPB–20 band number 7 of 1300 bp is a characteristic one in case of *C. epilinum* and in case of *G. campestris* bands number 4 and 15 of 1800 and 600 bp. By using primer OPD-10 a characteristic band number 5 of 1200 bp in case of *C. epilinum*, band number 8 of 900 bp is characteristic one in case of *G. campestris* and band number 11 of 600 bp in case of *C. pedicellata.* Using primer OPE–19 a characteristic bands number 15 and 17 of 250 and 150 bp in case of *C. epilinum,* bands number 4, 9 and 11 of 1500, 700 and 564 bp in case of *G. campestris* and band number 8 of 800 bp in case of *C. planiflora.* Using primer OPZ–1 a characteristic band number 2 of 1500 in case of *C. epilinum*. Using primer, OPZ–3 show two common bands numbers 4 and 7 of 1700 and 1300 bp, while, a characteristic band number 8 of 1100 bp in case of *C. epilinum*, bands number 5, 13 and 14 of 1500, 600, and 500 bp in case of G. *campestris,* respectively and band number 17 of 250 bp in case of *C. pedicellata* and by primer OPZ–8 a characteristic two bands in case of *G. campestris* number 1 and 13 of 2322 and 700 bp, band number 3 of 1700 bp in case of *C. planiflora* and band number 4 of 1664 bp in case of *C. pedicellata*.

RAPD–PCR offers several advantages over other methods used for studying variability among population because it is not limited to a single locus but theoretically detects polymorphisms across the whole genome either coding or non coding regions. Different parts of the genome are evolved at different rates (Nei, 1987). Higher variability can be obtained by RAPD–fingerprints especially if the amplified DNA regions contain micro and/or mini satellites (Baruffi *et al.*, 1995). The extensive polymorphisms detected among individuals indicate that RAPD–PCR can probably be used in a variety of ways for some species to measure clonally diversity among populations and determine the degree of relatedness among individuals and races.

Random Amplified Polymorphic DNA (RAPD) has been successfully applied to plants to develop genetic profiles. It has become an important tool in diverse fields of

plant population research e.g. the study of breeding systems, genetic relationships between or within species and populations, assessment of gene flow and gene identification (Epplen & Lubjuhn, 1999; Virk *et al.*,1995 and William *et al.*, 1990). In taxonomy and systematic, species–specific RAPD markers could be an invaluable tool for species verification and establishing the status of organisms of controversial taxonomy.

A major limitation in the use of the RAPD–PCR technique is that, most of the polymorphisms revealed by the RAPD–PCR segregate as dominant markers. Another drawback of RAPDs could be the lack of reproducibility of some minor bands in different runs. This problem can be avoided by considering only the major reproducible bands (Williams *et al.*, 1990). The risk of misinterpretation in RAPD analysis if different RAPD fragments had similar size, can be minimized by the use of several RAPD–primers so that the genetic analysis are based on large number of RAPD markers. Therefore in this investigation 12 arbitrory primers had been used.Eight of the primers used contained C and G residues at the beginning site 3' end. These nucleotides form more stable pairing configuration in double stranded DNA. Also each used primer contained C & G content ranging from 60 to 70%, which promote successful amplification. Table (5) shows that *Cuscuta pedicallata* has a similarity index with *C. planiflora* (42.0 %) and these expressed as the similar in some morphological characters of the two species. Low similarity index (8.0 %) between *C. pedicellata, C. epillinum* and *G. campestris* respectively. *C. planiflora* has low similarity ibdex (8.0 %) with *C. epillinum* (which has a specific host "flax") and no similarity at all with *C. campestris. C. epilinum* has low similarity index with other *Cuscuta* species (8.0 %). *C. campestris* has a specific index with other species; it has low similarity index with *C. pedicellata* and *C. epillinum* (8.0 %) and no similarity with *C. planiflora* (0.0 %). According to the similarity index (Table 5), the presence of a specific protein band number 3 (Table, 4) in addition to the morphological characters (Fig. 1) and the shape of stigma (Fig. 2), *C. campestris* can be separated from the other species of Genus *Cuscuta* and considered to belong to Genus *Grammica.*

In conclusion, the goal of the present study has been achieved by two different criteria, protein SDS–PAGE and RAPD–PCR analysis. Results show that *C. epilinum* differs by 14 characteristic RAPD-DNA bands than the three other species; *C. pedicellata, C. planiflora* and *G. campestris. G. campestris* which grows every where and parasitizes on any crop or weed, is characterized by golden yellow color and grow

in all seasons. Protein profile shows 16 characteristic bands differ than the other three species. *C. pedicellata* and *C. planiflora* belonging to genus *Cuscuta* are considered to be a complex group (Chrtek and Osbronova, 1991). According to the similarity in flower shape and size, calyx frequency and only differs in the ratio between stigma and style to the length of ovary where in *C. planiflora* the ovary is shorter. RAPD-DNA banding profiles show the difference between the two species (*C. pedicellata* and *C. planiflora*) in 25 bands. Results of protein and DNA analysis showed that dodder has polymorphic characters on molecular level suggest that dodder has two genera; *Gramica* '*G. campestris*' and *Cuscuta* '*C. pedicellata*, *C. planiflora* and *C. epilinum*'.

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Table (2): Electrophoretic protein banding pattern analysis by SDS-PAGE for three *Cuscuta* speices and *Grammica campestris* as indicated by using Gel-Pro V.3.0 computer analysis system.

Lanes	1	2		3		4		5	
sample	Marker	*C. pedicellata*		*C. planiflora*		*G. campestris*		*C. epilinum*	
Rows	(Mol. Wt.)	P.C.	Band %	P.C.	Band %	P.C.	Band %	P.C.	Band %
R_1	90.161	0	-	0	-	1	0.76	0	-
R_2	86.078	0	-	0	-	0	-	1	0.471
R_3	77.257	0	-	1	1.07	0	-	0	-
R_4	76.073	1	1.81	0	-	0	-	0	-
R_5	72.628	2	1.27	2	0.762	2	1.25	2	0.953
R_6	65.185	1	1.11	0	-	0	-	1	0.761
R_7	59.416	2	2.57	2	1.46	2	0.824	2	1.41
R8	58.055	0	-	0	-	0	-	1	2.39
R_9	57.608	0	-	0	-	1	2.36	0	-
R10	56.726	0	-	0	-	0	-	1	6.15
R11	55.0	2	5.12	2	6.82	2	6.86	2	4.08
R12	50.721	1	4.04	0	-	0	-	1	3.13
R13	46.774	2	2.81	2	2.18	2	1.34	2	5.21
R14	43.135	1	1.56	1	1.14	1	1.82	0	-
R15	41.546	1	1.45	1	0.936	0	-	1	1.87
R16	40.250	2	0.576	2	0.333	2	0.599	2	1.26
R17	39.739	0	-	0	-	0	-	1	1.49
R18	38.968	1	0.191	1	0.645	0	-	0	-
R19	37.962	1	0.767	1	2.11	0	-	0	-
R20	34.87	2	6.81	2	3.25	2	2.24	2	3.16
R21	32.03	1	3.53	0	-	1	3.53	0	-
R22	31.821	1	5.92	1	5.5	0	-	0	-
R23	30.392	2	2.48	2	6.28	2	2.36	2	3.58
R24	29.614	0	-	1	3.4	0	-	0	-
R25	28.662	2	0.324	2	1.75	2	7.79	2	10.1
R26	27.741	0	-	0	-	1	6.52	0	-
R27	27.025	0	-	0	-	0	-	1	2.08
R28	26.500	1	0.207	1	0.164	0	-	0	-
R29	21.325	1	2.25	0	-	0	-	1	1.59
R30	19.650	1	2.72	0	-	0	-	0	-
R31	18.255	2	5.29	2	3.29	2	2.83	2	4.44
R32	16.147	1	2.89	1	4.25	0	-	1	1.48
R33	14.638	2	4	2	3.1	2	2.1	2	1.28
Sum		23		19		15		20	

P.C = band presentation code (0) absent, (1) present and (2) present in all species.

Table (3): Results of DNA analysis of three *Cuscuta* species and *G.campestris* using different primers as indicated by gel documentation system Gel. Pro. V.3 computer program.

Primers	Lanes	1	2		3		4		5	
	sample	Marker	*C. pedicellata*		*C. planiflora*		*G. campestris*		*C. epilinum*	
	Rows	(Mol. Wt.)	P.C.	Band %	P.C.	Band %	P.C.	Band %	P.C.	Band %
OPA-1	R_1	2027	1	0.202	1	2.15	0	-	0	-
	R_2	1500	0	-	0	-	1	9.27	0	-
	R_3	1400	1	7.11	1	7.03	0	-	0	-
	R_4	1200	1	36.1	1	27.1	1	7.64	0	-
	R_5	1100	0	-	1	11.3	0	-	1	27.1
	R_6	1000	1	9.78	0	-	0	-	1	2.17
	R_7	900	2	7.29	2	6.71	2	27.7	2	1.24
	R8	800	1	2.52	1	2.8	1	5.7	0	-
	R9	700	0	-	0	-	1	0.721	1	17.3
	R10	600	1	0.235	1	1.4	1	1.68	0	-
	R11	500	2	0.379	2	1.22	2	0.973	2	2.25
	Sum		10	-	10	-	9	-	7	-
OPB-7	R1	2072	1	0.749	0	-	1	1.56	0	0
	R_2	1500	1	1.16	0	-	1	1.46	1	0.653
	R_3	1400	1	0.799	0	-	1	2.84	1	2.25
	R4	1300	0	-	0	-	1	7.1	0	-
	R5	1200	1	2.31	1	2.87	1	4.45	0	-
	R6	1100	1	4.05	0	-	0	-	0	-
	R7	1000	0	-	1	1.11	1	7.73	0	-
	R8	900	1	2.39	1	2.63	0	-	1	9.31
	R9	800	1	3.29	0	-	0	-	1	16.5
	R10	700	2	6.93	2	2.92	2	5.67	2	14.5
	R11	600	0	-	0	-	1	31.3	1	9.82
	R12	564	1	31.6	1	30	0	-	1	4.83
	R13	500	1	1.45	0	-	1	1.11	1	2.57
	R_{14}	400	2	13	2	6.67	2	2.03	2	1.14
	R15	300	1	2.33	0	-	1	0.718	1	1.43
	R16	200	0	-	0	-	0	-	1	0.577
	Sum		14		9		13		13	
OPB-9	R1	3269	0	-	0	-	0	-	1	2.02
	R2	2352	0	-	0	-	1	2.79	1	3.58
	R3	1830	1	2.25	0	-	1	3.77	1	4.71
	R4	1500	1	7.97	1	8.07	0	-	0	-
	R5	1400	0	-	0	-	1	12.4	1	12.3
	R6	1300	1	2.49	0	-	1	8.87	1	8.64
	R7	1200	0	-	0	-	1	9.3	1	15.8
	R8	900	1	8.11	1	8.05	1	6.83	0	-
	R9	800	1	9.43	1	7.35	1	5.48	0	-
	R10	700	2	8.89	2	8.85	2	2.71	2	5.99
	R11	600	0	-	0	-	1	4.14	0	-
	Sum		7		7		9		8	
OPB-11	R_1	2027	0	-	0	-	1	2.84	1	4.8
	R_2	1700	1	9.36	1	6.73	1	7.5	0	-
	R3	1500	1	6.32	1	6.12	0	-	1	6.76
	R4	1400	2	4.37	2	3.78	2	3.25	2	3.56

	R5	1300	1	42.1	1	41.6	1	2.63	0	-
	R6	1200	0	-	0	-	1	5.13	0	-
	R7	1100	1	5.29	1	5.7	0	-	1	15.2
	R8	1000	1	1.79	1	2.77	0	-	1	6.73
	R9	900	0	-	0	-	1	9.42	0	-
	R10	800	0	-	0	-	1	2.91	0	-
	R11	600	0	-	0	-	1	26.8	0	-
	Sum		7		7		9		6	
	R1	6557	1	1.36	1	1.97	0	-	1	1.63
	R2	3700	1	2.48	1	1.86	0	-	1	1.95
	R3	2027	0	-	1	1.03	0	-	1	2.58
	R4	1500	2	20.2	2	10.5	2	2	2	18
	R5	1400	0	-	0	-	1	8.67	1	6.1
	R6	1300	1	5.17	1	2.41	0	-	1	2.98
	R7	1200	1	27.4	1	27	0	-	1	8.98
	R8	1100	0	-	0	-	1	19.2	1	12.2
OPB-18	R9	900	2	4.26	2	2.8	2	12.1	2	5.42
	R10	838	0	-	0	-	0	-	1	6.84
	R11	750	2	2.35	2	1.22	2	9.59	2	3.8
	R12	700	2	6.67	2	2.43	2	13	2	1.1
	R13	600	1	0.292	1	0.618	0	-	1	0.688
	R14	500	0	-	0	-	1	2.13	1	0.86
	R15	400	1	2.66	1	3.32	1	0.743	0	-
	R16	300	1	0.256	0	-	0	-	0	-
	Sum		15		15		12		18	
	R1	2850	1	1.56	1	0.651	0	-	0	-
	R2	2500	1	6.6	1	3.9	1	0.952	0	-
	R3	2072	1	1.61	0	-	1	1.22	1	2.19
	R4	2027	0	-	1	0.377	0	-	1	1.88
	R5	1800	0	-	0	-	1	35.9	0	-
	R6	1600	2	2.92	2	2.07	2	23.7	2	41.1
	R7	1500	0	-	0	-	0	-	1	2.07
OPB-19	R8	1400	0	-	0	-	1	25.3	0	-
	R9	1300	1	0.745	0	-	0	-	1	0.155
	R10	1100	0	-	0	-	1	2.38	1	0.682
	R11	1000	0	-	0	-	0	-	1	41.9
	R12	900	2	14.2	2	6.55	2	0.348	2	0.094
	R13	800	2	3.07	2	3.97	2	0.356	2	0.157
	R14	750	0	-	0	-	0	-	1	0.122
	R15	700	0	-	0	-	1	0.968	0	-
	R16	600	1	9.12	1	9.58	0	-	1	0.808
	R17	500	2	10.2	2	9.37	2	0.297	2	2.02
	R18	400	0	-	0	-	0	-	1	0.893
	R19	300	1	0.561	1	1.34	0	-	0	-
	Sum		14		13		14		17	
	R1	4421	1	1.1	1	0.648	0	-	0	-
	R2	3500	0	-	0	-	1	1.56	1	0.634
	R3	2027	0	-	0	-	1	6.36	1	1.39
	R4	1800	0	-	0	-	1	7.37	0	-
	R5	1600	0	-	0	-	0	-	1	0.341
	R6	1500	2	20.2	2	21	2	4.86	2	3.31
OPB-20	R7	1300	0	-	0	-	0	-	1	12.5
	R8	1200	1	9.24	1	8.08	0	-	0	-
	R9	1100	1	12.9	1	20.6	1	5.1	0	-

	R10	1000	1	2.09	0	-	1	10.5	1	2.26
	R11	900	1	34.6	1	23.3	1	36.8	0	-
	R12	800	2	2.19	2	0.058	2	4.15	2	4.94
	R13	750	2	1.13	2	0.974	2	0.12	2	0.731
	R14	700	2	9.68	2	8.94	2	16.3	2	28.2
	R15	600	0	-	0	-	1	0.444	0	-
	R16	500	2	1.03	2	0.812	2	1.31	2	31.5
	Sum		15		14		17		15	
	R1	2027	0	-	0	-	1	0949	1	4.95
	R2	1500	2	4.75	2	3.29	2	1.89	2	13
	R3	1400	1	31.8	1	21.3	1	2.98	0	-
	R4	1300	1	16.4	1	17.9	0	-	0	-
	R5	1200	0	-	0	-	0	-	1	2.69
OPD-10	R6	1100	2	9.25	2	6.48	2	36.7	2	5.61
	R7	1000	1	15.7	1	9.75	0	-	0	-
	R8	900	0	-	0	-	1	19.5	0	-
	R9	800	2	1.43	2	2.05	2	4.45	2	4.77
	R10	700	1	91.2	1	3	0	-	0	-
	R11	600	1	3.03	0	-	0	-	0	-
	Sum		11		10		9		8	
	R1	2072	1	2.8	1	1.84	0	-	0	-
	R2	2027	2	6.04	2	3.89	2	11.1	2	5.29
	R3	1700	1	7.99	1	5.3	0	-	1	2.41
	R4	1500	0	-	0	-	1	11.4	0	-
	R5	1400	1	5.57	1	6.55	0	-	1	14.4
	R6	1100	0	-	0	-	1	23.2	1	3.11
	R7	1000	1	14	1	14.1	0	-	0	-
	R8	800	0	-	1	5.26	0	-	1	3.4
OPE-19	R9	700	0	-	0	-	1	19.7	0	-
	R10	600	1	9.56	1	9.7	0	-	1	3.17
	R11	564	0	-	0	-	1	3.79	0	-
	R12	500	1	14.5	1	17.5	1	1.43	0	-
	R_{13}	400	2	6.93	2	7.87	2	0.846	2	5.9
	R_{14}	300	2	0.487	2	1.33	2	1.01	2	0.947
	R_{15}	250	0	-	0	-	0	-	1	0.356
	R_{16}	200	2	0.552	2	.872	2	0.38	2	0.305
	R_{17}	150	0	-	0	-	0	-	1	0.312
	Sum		14		15		13		15	
	R_1	1700	0	-	1	3.49	1	7.97	1	2.71
	R_2	1500	0	-	0	-	0	-	1	4.43
	R_3	1300	1	2.44	1	3.28	1	5.66	0	-
	R_4	1100	2	5.34	2	3.95	2	3.73	2	3.03
OPZ-1	R5	1000	1	3.78	1	3.38	0	-	0	-
	R6	900	0	-	0	-	1	5.9	1	4.3
	R7	800	1	1.91	1	3.03	0	-	0	-
	R8	700	1	2.19	1	4.07	1	11	0	-
	R9	600	1	14.3	1	12.6	0	-	0	-
	R10	500	1	3.22	1	6.08	0	-	1	17.3
	Sum		8		9		6		5	
	R1	3251	1	1.62	1	1.5	1	2.41	0	-
	R2	2322	1	1.82	1	1.08	1	2.33	0	-
	R3	2027	1	2.46	1	1.14	0	-	0	-
	R4	1700	2	20.5	2	21.2	2	9.89	2	8.64
	R5	1500	0	-	0	-	1	25.2	0	-

	R6	1400	1	5.18	1	4.94	0	-	1	9.17
	R7	1300	2	6.58	2	6.67	2	7.59	2	9.35
	R8	1100	0	-	0	-	0	-	1	4.77
OPZ-3	R9	1000	1	5.99	0	-	0	-	1	10.4
	R10	900	1	8.7	1	18.5	0	-	0	-
	R11	800	1	2.23	0	-	0	-	1	6.23
	R12	700	1	1.66	1	2.77	0	-	1	3.48
	R13	600	0	-	0	-	1	3	0	-
	R14	500	0	-	0	-	1	0.217	0	-
	R15	400	1	0.591	1	2.46	0	-	0	-
	R16	300	1	0.574	1	1.89	1	2.66	0	-
	R17	250	1	0.54	0	-	0	-	0	-
	Sum		15		12		10		9	
	R1	2322	0	-	0	-	1	1.54	0	-
	R2	1827	2	3.28	2	1.7	2	4.67	2	4.76
	R3	1700	0	-	1	3.1	0	-	0	-
	R4	1664	1	5.45	0	-	0	-	0	-
	R5	1500	1	6.94	1	5.21	0	-	0	-
	R6	1310	0	-	0	-	1	13.4	1	11.1
	R7	1200	2	2.92	2	1.91	2	9.82	2	10.4
	R8	1100	1	3.14	1	1.49	0	-	1	6.75
	R9	1040	0	-	0	-	1	3.93	0	-
	R10	950	1	4.25	1	3.55	0	-	0	-
	R11	880	0	-	0	-	1	5.19	1	8.6
	R12	800	1	6.82	1	8.54	0	-	1	6.27
	R13	700	0	-	0	-	1	4.56	0	-
	R14	600	0	-	0	-	1	17.3	1	5.99
	R15	500	1	3.16	1	5.2	1	3.78	0	-
	R16	400	1	0.686	1	1.51	0	-	0	-
	Sum		11		11		10		8	

P.C = band presentation code (0) absent, (1) present and (2) present in all species.

Table (4): Comparison between the total bands numbers for the 4 studied species as regards of SDS-PAGE protein and 12 RAPD-DNA primers.

	C. pedicellata			*C. planiflora*			*C. epillinum*			*G. campestris*		
Bands	T.	G.	Sp.	T.	G.	Sp.	T.	G.	Sp.	T.	G.	Sp.
Protein	23	10	2	19	10	2	20	10	5	15	10	3
Decamer Oligonuclutide Primer												
OPA-1	10	2	0	10	2	0	7	2	0	9	2	1
OPB-7	14	2	1	9	2	0	13	2	1	13	2	1
OPB-9	7	1	0	7	1	0	8	1	1	9	1	1
OPB-11	7	1	0	7	1	0	6	1	0	9	1	4
OPB-18	15	4	1	15	4	0	18	4	1	12	4	0
OPB-19	14	4	0	13	4	0	17	4	4	14	4	3
OPB-20	15	5	0	14	5	0	15	5	2	17	5	2
OPD-10	11	3	1	10	3	0	8	3	1	9	3	1
OPE-19	14	4	0	15	4	0	15	4	2	13	4	3
OPZ-1	8	1	0	9	1	0	5	1	1	6	1	0
OPZ-3	15	2	1	12	2	0	9	2	1	10	2	3
OPZ-8	11	2	1	11	2	1	8	2	0	10	2	3
Total	141	31	5	132	31	1	129	31	14	131	31	22

T: Total band number; G: General band number and Sp.: Specific band number.

Table (5): The similarity coefficient according to Sörenson (1948) for the total band number for *Cuscuta* and *Grammica* species.

	C. pedicellata	*C. planiflora*	*C. epillinum*	*G. campestris*
C. pedicellata		42.0	8.0	8.0
C. planiflora	42.0		8.0	0.0
C. epillinum	8.0	8.0		8.0
G. campestris	8.0	0.0	8.0	

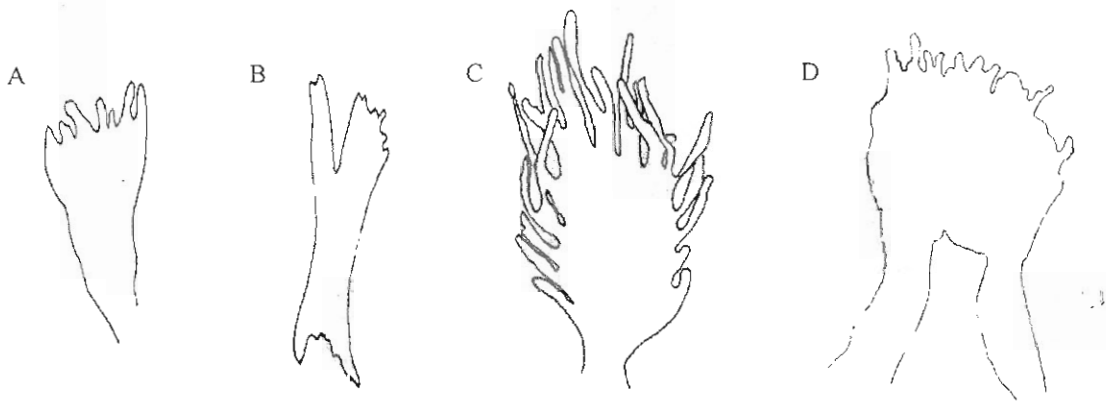


Figure (1) : Scale structure :

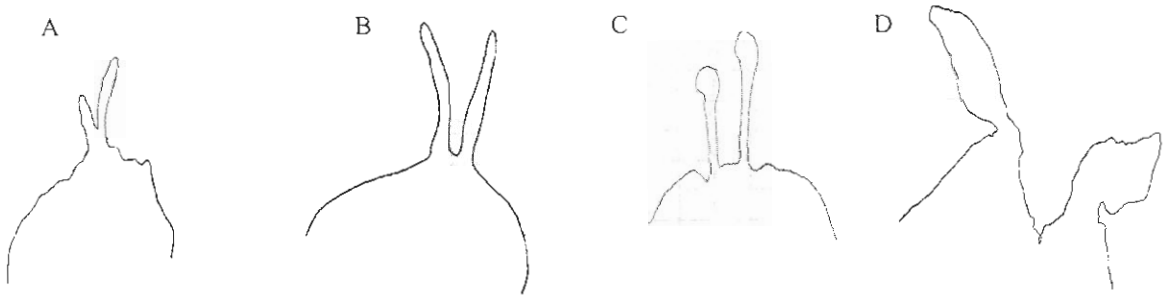


Figure (2) : Gynecium structure

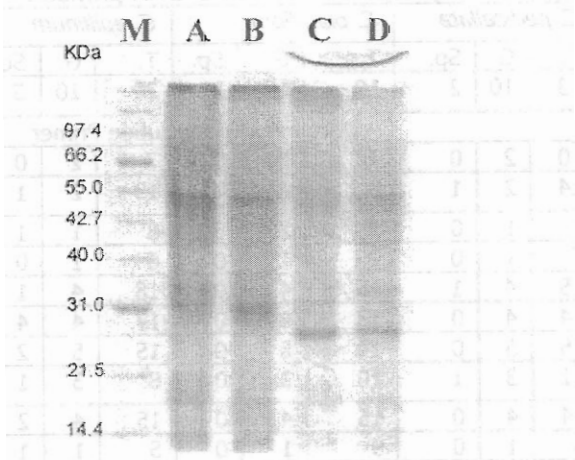
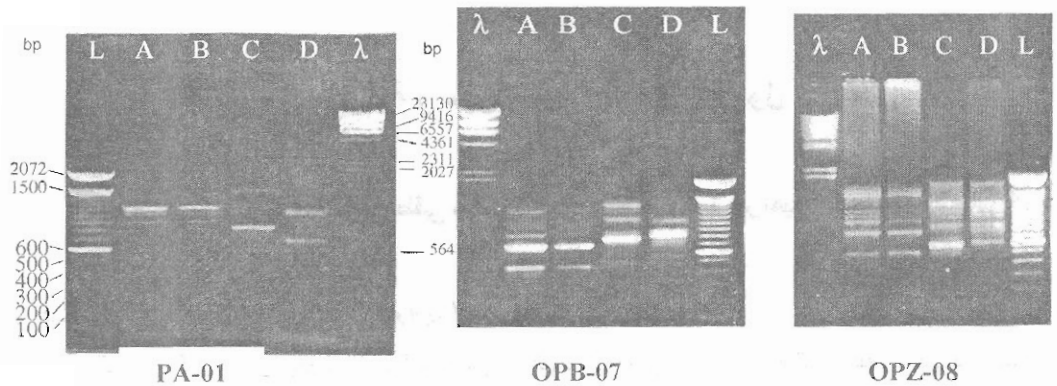


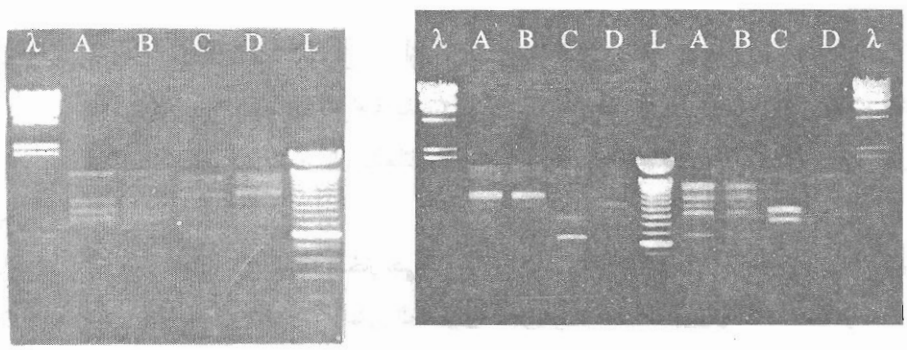
Figure (3): Elecetrophoretic banding analysis pattern for *Cuscuta* and *Grammica* species.

A: *C. pedicellata* B: *C. planiflora* C: G. *campestris* D: *C. epilinum*

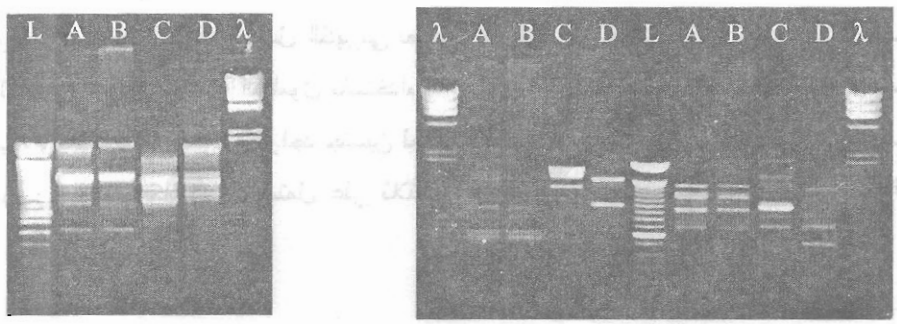
Figure (4): PCR-RAPD pattern for *Cuscuta* and *Grammica* species.



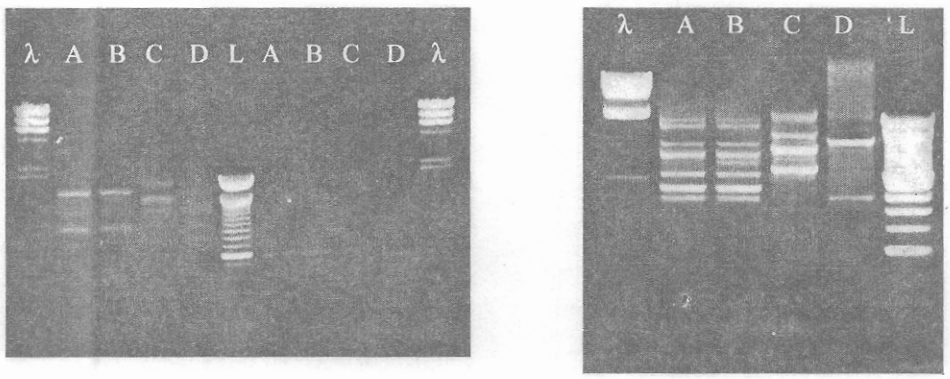
PA-01 OPB-07 OPZ-08



OPB-9 OPB-11 OPD-10



OPB-18 OPB-19 OPB-20



OPZ-03 OPZ-01 OPE-19

دراسات جزيئية و مورفولوجيه لجنس الحامول فى مصر

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**المعمل المركزى لبحوث الحشائش، مركز البحوث الزراعية، وزارة الزراعة، الجيزة، مصر*

الحامول جنس نباتى متطفل غير مورق ذو أزهار صغيرة الحجم متجمعة فى رؤوس. يتطفل الحامول على عوائل عديدة من بين النباتات الراقية باستثناء 'كاسكوتا ابيلينوم' الذي ينمو على نبات الكتان فقط . هذا ويمد الحامول ممصاته للإتصال بالعائل للحصول على أحتياجاته الغذائية لذا ينمو بدون جذور عادية.

أيضا يظهر الحامول تباينا فى شكله الخارجي على عوائله المتنوعة كما أن تميزه مورفولوجيا ليس كاملا . لذلك أجريت بعض التجارب على المستوى الجزيئى بالنسبة للبروتينات المستخرجة من بذور الحامول وكذا الحمض النووي (د ن ا) لعينات غضة من سيقان الحامول محل الدراسة بمصر خلال الفترة من ١٩٩٨حتى ٢٠٠٢.

ولقد أوضحت نتائج تجارب الفصل الكهربى بطرق الشرائط لبروتينات بذور الحامول المستخدمة وكذا الحمض النووى لعينات الحامول باستخدام ١٢ بادئ تباين خصائص الحامول على المستوى الجزيئى، وخلصت الدراسةالى تواجد جنسين لعائلة الحامول بمصر هما : جنس جراميكا 'ج. كامبستريس' وجنس كاسكوتا ويشتمل على ثلاثة أنواع هى (ك. بيديسيلاتا، ك. بلانيفلورا، وك. ايبيلينوم).