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Biochemical and molecular genetic characterization of four species of family Polygonaceae in Egypt.

Magda I. Soliman, Yasser A. El-Amier and Eman M. Fayed.
Botany Department, Faculty of Science, Mansoura University, Egypt..

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Abstract The aim of the present study is to investigate the interrelationships between the four studied taxa namely *Persicaria lanigera* (R. Br.) Sojak, *P. lapathifolia* (L.) Gray, *P. salicifolia* (Brouss. ex Willd.) Assenov and *Polygonum equisetiforme* Sm. collected from ten different accessions in Egypt belonging to family Polygonaceae. Biochemical studies include protein profile using polyacrylamide gel electrophoresis (SDS-PAGE) technique. The electrophoretic analysis revealed the presence of seventeen bands of molecular weight ranging from 11.00 to 155.00 KDa. The highest numbers of bands were 11 observed in *Pe.lap5* collected from Kafr-El Hataba Nile region-Dakahlya Governorate and *Pe.sal6* collected from El-Sahel canal Sherbin- Dakahlya Governerate whereas the lowest numbers of bands were 4 recorded in *Pe.sal7* collected from El-Westani Drainge canal Damietta Governorate. DNA polymorphism of selected taxa was studied using different marker systems: Random amplified polymorphic DNA (RAPD) and Inter Simple Sequence Repeat (ISSR). Five primers used in RAPD and ISSR techniques separately the size of amplified fragment product range was (320-1150bp) and (150-2130bp) for RAPD and ISSR respectively. The percentage of polymorphism produced by each primer was different from one primer to the other. The results obtained from biochemical and molecular genetic information revealed a remarkable discrimination between taxa under study.

Introduction

The Polygonaceae is cosmopolitic to temperate regions (Täckholm, 1974) and (Boulos, 1999).The family is taxonomically divided in two subfamilies, Polygonoideae and Eriogonoideae. The subfamily Polygonoideae includes five tribes: Calligoneae, Fagopyreae, Persicarieae, Polygoneae and Rumiceae (Burke *et al.* 2010). There are 46 genera with 1100 species in the world (Boulos, 1999). In Egypt, The family is represented by 28 species belonging to seven genera, if *Persicaria* is considered as a section within *Polygonum* (Davis, 1967; Täckholm, 1974; Zohary, 1966 and Meikle, 1985), or eight genera after they were separated into two genera (Boulos, 1999 and Chaudhary, 1999).

Morphologically, Persicarieae is distinguished from the other major tribes Polygoneae by having non-dilated filamentous stamens with number recorded mostly in the outer whorl and mostly rectangular to elongated tepal epidermal cell (Decraene & Akeroyed, 1988 and Decraene *et al.*, 2000).

The genus Polygonum L. (Polygonaceae), well-known as Persicaria or knotweed, is an important group of medicinal plants used for various purposes (Soodabeh, *et al.*, 2011).

Polygonum species has been reported in folk medicine for the treatment of atherosclerosis, hyper-tension, cough, suppurative dermatitis and gonorrhea (Yi *et al.* 2007). The genus *Polygonum* is introduced as a source of numerous phenolic compounds, flavonoids, anthraquinonones, stillbenes and tannins (Lin *et al.* 2003). Flavonoides are the most common compounds in *Polygonum* species and have previously been used as chemotaxonomic markers in the systematics of Polygonaceae plants (Datta *et al.*, 2000). In addition the use of *Polygonum* species as a food for man and animals has been reported in different parts of the world (Zahran and Willis, 1992).

Seed protein electrophoresis has been successfully used to define species relationships in various groups of plants (Ladizinsky, 1975). One of the biochemical markers is SDS-PAGE; it has been widely used due to its simplicity and effectiveness for estimating genetic differentiation. Variation in SDS-PAGE of seed protein banding patterns has successfully been used to differentiate between species in a number of genera for example, some species of family Malvaceae (Rizk and Soliman, 2014) and *Mesmbryanthemum* species (Soliman *et al.*, 2014).

In recent years, a number of PCR based DNA markers have been developed to evaluate genetic variation at intraspecific and interspecific levels (Wolf and Liston., 1998). Randomly amplified polymorphic DNA (RAPD) technique has been used in many different applications involving the detection of DNA sequence polymorphisms ,(Carlson *et al.*, 1991 and Reiter *et al.*, 1992) and to assess the genetic diversity (Abdel-Tawab *et al.*, 1998 and 2001). $RAPD_S$ and DNA profiles are used to study the genetic variation and relatedness among the cultivars, construct phylogenies and calculate the genetic distances.

Major limitation of RAPD method was the low reproducibility. ISSR markers overcome this limitation (Zietkiewicz *et al.*, 1994; Gupta *et al.*, 1994; Wu *et al.*, 1994 and Meyer *et al.*, 1993), as the advantage of ISSR over RAPD is its being more reproducible (Fernandez *et al.*, 2002 and Greene *et al.*, 2004). The first study employing Inters Simple Sequence Repeat ($ISSR_S$) was published in 1994 by Zietkiewicz *et al.* (1994). Giedre *et al.* (2011) confirmed the proposition that ISSR and RAPD techniques are useful in clover genomic diversity studies and breeding programs.

Therefore, in the present study SDS-PAGE, RAPD and ISSR markers have been used in order to determine genetic variation and relationships of four species of Polygonaceae which were collected from different ten accessions of Egypt. This work is very important to document in gene banks for sustainable conservation of plant genetic resources.

Materials and Methods

Plant materials: Ten accessions of four plant species of family Polygonaceae inhabited in Egypt were collected from their natural habitats. Table (1) showing their localities. Identification nomenclature of studied species was according to Täckholm (1974) and Boulos (2000).

Table (1): Names and localities of ten accessions of the four studied taxa collected from Egypt.

No.	Taxa	Code	Locality
1	*Persicaria lanigera* (R. Br.) Sojak	(*Pe.lan1*)	EL-Adlia EL-Nile (EL-Salaam)-Damietta Governorate.
2		(*Pe.lan2*)	El-Motamadya village –El-Gharbya Governorate.
3	*Persicaria lapathifolia* (L.) Gray	(*Pe.lap3*)	Kafr El-Hataba-Nile region (Talkha) Dakahlya Governorate.
4		(*Pe.lap4*)	EL-Adlia EL-Nile (EL-Salaam)-Damietta Governorate.
5		(*Pe.lap5*)	Om-Reda –Damietta Governorate
6	*Persicaria salicifolia* (Brouss. ex Willd.)	(*Pe.sal6*)	EL-Sahel canal –Sherbin-Dakahlya Governorate.
7		(*Pe.sal7*)	EL-Westani Drainge canal- Dakahlya Governorate.
8		(*Pe.sal8*)	Kafr EL-Tawila (Talkha) Dakahlya Governorate.
9	*Polygonum equisetiforme* Sm.	(*Po.equ9*)	Baltim- Kafr EL- Sheikh Governorate.
10		(*Po.equ10*)	Gamasa coast Dakahlya Governorate.

Protein analysis

Electrophoresis analysis of seed proteins followed the method for discontinuous SDS-PAGE technique of Laemmli (1970).

DNA Extraction: Genomic DNA was extracted from fresh young leaves of the studied accessions according to Dellaporta *et al.* (1983).

Random Amplified polymorphic DNA (RAPD-DNA):

Five primers were used to generate RAPD markers according to Williams *et al.* (1990).The sequence of these primers is given in Table (2).

Inter-Simple Sequence Repeats DNA (ISSR-DNA):

Five primers were tested to amplify the isolated DNA following (Zietkiewicz *et al.*, 1994). These primers are listed in Table (2).The gel was photographed, scanned and analyzed using Gel Doc 2000 Bio-Rad system. The presence or absence of each band was treated as a binary character in a data matrix (coded 1 and 0 respectively). Data analyses were performed using the SYSTAT version 7.0 program (Wilkinson, 1997).

Table (2): List of primers and their composition used in RAPD and ISSR analysis.

Sequence 5'to 3'

No.	Primer code	RAPD	No.	Primer code	ISSR
1	OP O9	5-TCCCACGCAA-3	1	HB9	$(GT)_6$ GG
2	OP O10	5-TCAGAGCGCC-3	2	HB10	$(GA)_6$ CC
3	OP O11	5-GACAGGAGGT-3	3	HB11	$(GT)_6$ CC
4	OP O12	5-CAGTGCTGTG-3	4	HB13	$(GAC)_3$ GC
5	OP O13	5-GTCAGAGTCC-3	5	HB14	$(CTC)_3$ GC

Results and Discussion

The use of seed proteins and its metabolites as a taxonomic marker is well established (Toms and Western, 1971). Protein electrophoresis analysis provides taxonomic evidence for separation of taxa at the species and variety levels as shown for *Zygophyllum* L. (Khafagi, 2003), *Tribulus* (Mohamed, 2006) and *Cleome* (Mohamed, 2009).

Polyacrylamide gel electrophoresis (PAGE) for revealing protein and isozyme polymorphism was used for identification of species and subspecies and varieties in a number of plant genera (Adrianse *et al.*, 1969, Boulter *et al.*, 1970 and Badr, 1995).

The electrophenogram (Fig. 1) and seed protein attributes illustrated in Table (3). The electrophoretic analysis revealed the presence of seventeen bands of molecular weight ranging from 11.00 to 155.00 KDa. The highest number of bands 11 was observed in *Pe.lap*5 and *Pe.sal*6 collected from Om-Reda –Damietta Governorate and from EL-Sahel canal –Sherbin-Dakahlya Governerate respectively. On the other hand the lowest numbers of bands were recorded in *Pe.sal*7 collected from EL-Westani Drainge canal-Dakahlya Governorate. The highest percentage of polymorphism 64.70 % recorded in *Pe.sal5* and *Pe.sal*6 collected from Om-Reda – Damietta Governorate and from EL-Sahel canal –Sherbin-Dakahlya Governorate respectively. The percentage of polymorphism was given in Table (3). The band of molecular weights 120 KDa recorded only in *Persicaria lanigera* (1 and 2). So that this band could be used as positive marker in contrast to band of molecular weight 65 KDa that is present in all accessions except in the two sites of *Persicaria lanigera* so that this band is considered a negative marker to this species. The band of molecular weight 11.00 KDa was found in all accessions and absent in site 1 of *Pe.lan1* so that this band could be used as negative marker.

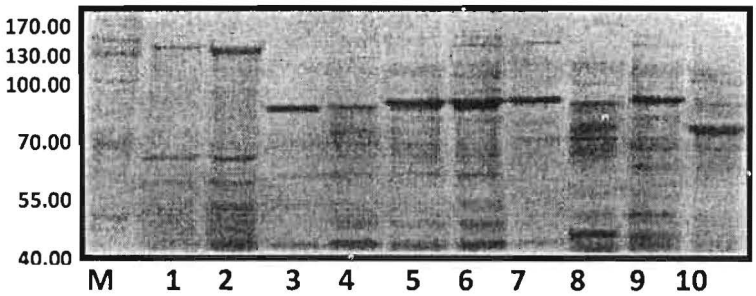

Fig. (1): Polyacrylamide gel illustrating seed proteins bands of some taxa belonging to family Polygonaceae. (M) Marker. For accessions names see Table (1).

Table (3): Seed protein attributes of some species of family Polygonaceae collected from different accessions. For accessions names see Table (1).

Band No.	MW. (KDa)	1	2	3	4	5	6	7	8	9	10
1	155	-	-	-	-	+	+	-	-	-	-
2	145	-	-	-	+	+	+	-	-	+	-
3	120	+	+	-	-	-	-	-	-	-	-
4	118	+	+	-	-	-	-	+	+	-	-
5	116	-	+	-	-	+	+	-	+	+	+
6	114	-	+	-	-	+	+	-	+	-	+
7	95	-	-	-	+	+	+	-	+	-	-
8	65	-	-	+	+	+	+	+	+	+	+
9	60	-	-	+	-	-	-	-	-	+	-
10	45	-	-	+	+	-	-	+	+	-	+
11	31	+	+	+	-	+	+	-	-	+	-
12	29	+	+	+	+	+	+	-	-	-	-
13	27	+	+	-	-	-	-	-	-	-	-
14	26	+	+	-	+	+	+	-	+	+	+
15	22	-	-	-	-	-	-	-	+	+	+
16	20	-	-	-	+	+	+	-	-	-	-
17	11	-	+	+	+	+	+	+	+	+	+
Total		6	9	6	8	11	11	4	9	8	7
% of polymorphism		35.29	52.94	35.29	47.05	64.70	64.70	23.52	52.94	47.05	41.17

The results of DNA profiles using five RAPD primers (OPo9, OP10, OPo11, OPo12 and OPo13) and five ISSR primers (HB9, HB10, HB11, HB13 and HB14) are illustrated in plate (1). The range of band products, numbers of common and polymorphic bands produced by different primers are shown in Tables (4 and 5). The studied accessions of *Persicaria* and *Polygonum* revealed a total of 44 bands ranging in size from 320 to 1150 bp using five RAPD primers. The result indicated that primer OPo9 produced two bands of molecular weight 560 and 410 bp respectively these bands were considered as unique bands for accession 10 (*Po.equ10*) collected from Gamasa coast Dakahlya Governorate and the other band was unique for site1 (*Pe.lan1*) collected from EL-Adlia EL-Nile (EL-Salaam)-Damietta Governorate. Considering the primer OPo13 a band of M.wt 380 is a unique band for site1 (*Pe.lan1*) collected from EL-Adlia EL-Nile (EL-Salaam)-Damietta so it can be regarded as positive marker for this accession. Band of M.wt 450 was present in all accessions except site6 (*Pe.sal6*) collected from EL-Sahel canal–Sherbin-Dakahlya Governorate so it could be regarded as a negative marker for this site. It is found that a producible band of molecular weight 350 bp was present in all sites but absent in site10 (*Po.equ*10) collected from Gamasa coast Dakahlya Governorate this could be used a negative marker. These results indicated that DNA analysis using RAPD technique may be considered as a good tool for DNA fingerprinting, detecting the genetic variations and identifying of *Persicaria* and *Polygonum* species which had been collected from different accessions. This made a good conformation to the previous studies on DNA amplification fingerprinting as the randomly amplified polymorphic (RAPD) technique has been used in many different applications such as the detection of DNA sequence polymorphism (Carlson *et al.*, 1991) and Reiter *et al.*, 1992), identification of varieties (He *et al.*, 1992) and the assessment of genetic diversity (Abdel-Tawab *et al.*, 2001; Soliman *et al.*, 2012 and Sara, 2013). Moreover, this technique has been found very useful for genetic fingerprinting and for facilitating of the positional cloning of the genes (Waldron *et*

al., 2002) as well as cultivar identification and mapping purposes (Zhang *et al.*, 2003).

Concerning ISSR primers a total of 44 bands ranging in size from 150 to 2130 bp, among which 8 were common in all accessions and 36 were polymorphic bands. The HB9 primer has generated two bands of molecular size 850 and 240 bp both are present in all accessions except in site8 (*Pe.sal8*) and site10 (*Po.equ10*) respectively. PCR amplification of DNA using HB10 primer produced two bands of molecular size 1050 and 570 bp both are unique bands for site8 (*Pe.sal8*) and site6 (*Pe.sal6*) collected from Kafer EL-Tawila (Talkha) Dakahlya Governerate and EL-Sahel canal–Sherbin-Dakahlya Governerate respectively so it can be recorded as positive markers for both accessions. Bands with molecular weight 290bp produced by primer HB11 this also is the unique band for site8 (*Pe.sal8*) collected from Kafr EL-Tawila (Talkha) Dakahlya Governorate, it could be regarded as positive marker for this accession. The primer HB13 produced 3 unique bands with M.wt 1230, 1200 and 310 two of them were present in site2 (*Pe.lan2*) collected from El-Motamadya village –El-Gharbya Governorate and band 310 was unique for site3 (*Pe.lap3*) collected from Kafer El-Hataba-Nile region (Talkha) Dakahlya Governorate so each of these bands could be considered as a positive marker for these accessions respectively. Finally primer HB14 produce band with molecular weight 980bp absent in all sites except site1 (*Pe.lan1*) collected from EL-Adlia EL-Nile (EL-Salaam)-Damietta Governorate considered as positive marker, in contrast to bands with M.wt 860 and 710bp both were present in all accessions but absent in site5 (*Pe.lap5*) so we they could be considered as a negative marker for this accession as shown in Plate (1) and Table (4).

According to Guo *et al.* (2009), ISSR has several advantages including high annealing temperature and repetition and lower cost. This has been widely used with the medicinal plant species. ISSR might be a good tool for DNA fingerprinting of plant cultivars (Shi *et al.*, 2010) and also this technique has offered a fast and practical way to detect interspecific hybrids early in the breeding programme (Goldman, 2008). Moreover, ISSR has been used to assess the genetic diversity in plant species (Emel, 2010 and Gajera *et al.*, 2010).

Plate (1): DNA polymorphism based on RAPD-PCR and ISSR-PCR analysis of *Persicaria* and *Polygonum* accessions in Egypt.

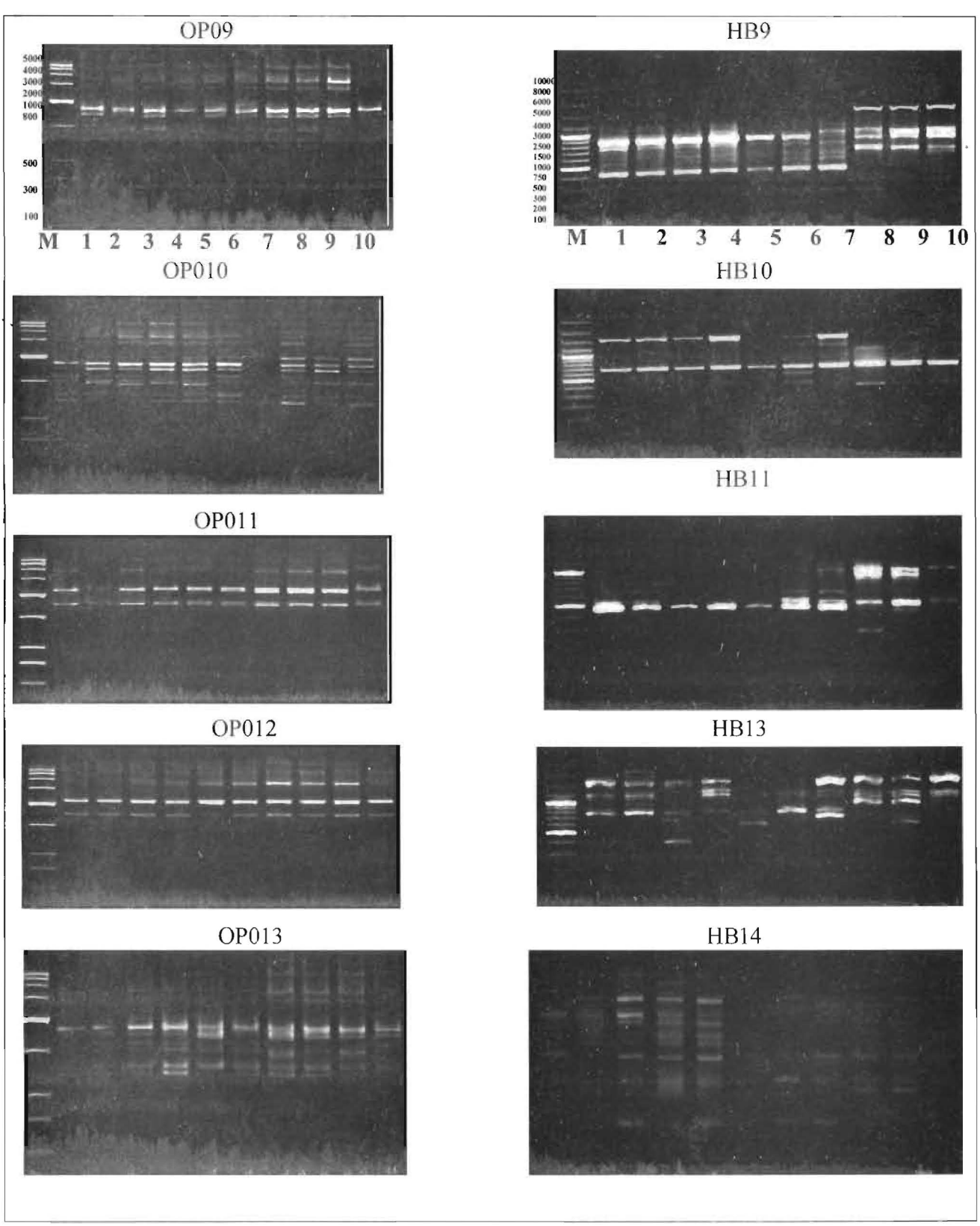

Table (4): RAPD and ISSR profiles of different genotypes in *Persicaria* and *Polygonum* using five primers. For accessions that shown in Table 1.

RAPD

Band no.	MW (Bp)	Genotype									
		Pe.km1	Pe.km2	Pe.kp3	Pe.kp4	Pe.kp5	Pe.mi6	Pe.mi7	Pe.mi8	Po.ep9	Po.ep10
OP09											
1	990	1	1	1	1	1	1	1	1	1	1
2	740	1	1	1	1	1	1	1	1	1	1
3	690	1	1	1	1	1	1	1	1	1	1
4	580	1	1	1	1	1	1	1	1	1	1
5	560	0	0	0	0	0	0	0	0	0	1
6	540	1	1	1	1	1	1	1	1	1	1
7	460	1	0	0	0	0	1	1	1	1	1
8	410	1	0	0	0	0	0	0	0	0	0
9	385	0	1	1	1	1	1	1	1	1	1
10	350	1	1	1	1	1	1	1	1	1	1
11	320	1	1	0	0	0	0	1	1	1	1
Total		9	8	7	7	7	8	9	9	9	10
OP010											
1	980	0	1	1	1	1	0	0	1	1	1
2	840	0	0	0	0	0	0	0	1	1	1
3	810	1	1	1	1	1	1	1	1	1	1
4	750	1	1	1	1	1	1	1	1	1	1
5	590	1	1	1	1	1	1	1	1	1	1
6	560	1	1	1	1	1	1	1	1	1	1
7	490	1	1	1	1	1	1	1	1	1	1
8	450	1	1	1	1	1	1	1	1	1	1
9	410	1	1	1	1	1	1	1	1	1	1
10	385	0	0	1	0	1	0	0	0	0	0
11	360	1	1	1	1	1	1	1	1	1	1
12	340	1	1	1	1	1	1	1	1	1	1
Total		9	10	11	10	11	9	9	11	11	11
OP011											
1	980	1	1	1	1	1	1	1	1	1	1
2	850	1	0	1	0	1	1	1	1	1	1
3	790	1	0	1	1	1	1	1	1	1	0
4	620	1	1	1	1	1	1	1	1	1	1
5	550	1	1	1	1	1	1	1	1	1	1
Total		5	3	5	4	5	5	5	5	5	4
OP012											
1	1150	0	0	0	0	0	0	1	1	1	0
2	1050	0	0	0	0	0	0	1	1	1	0
3	970	1	1	1	1	1	1	1	1	1	1
4	890	1	1	1	1	1	0	0	0	0	0
5	780	1	0	1	1	1	1	1	1	1	1
6	540	1	1	1	1	1	1	1	1	1	1
7	470	1	1	1	1	1	1	1	1	1	1
Total		5	4	5	5	5	4	6	6	6	4
OP013											
1	1050	0	0	1	0	1	1	1	1	1	1
2	830	1	1	1	1	1	1	1	1	1	1
3	780	1	1	1	1	1	1	1	1	1	1
4	490	1	1	1	1	1	1	1	1	1	1
5	450	1	1	1	1	1	0	1	1	1	1
6	430	1	1	1	1	1	0	0	1	1	1
7	410	0	1	1	1	1	0	1	1	1	1
8	380	1	0	0	0	0	0	0	0	0	0
9	350	1	1	1	1	0	1	1	1	1	0
Total		7	7	8	7	7	5	7	8	8	7

ISSR

Band no.	MW (Bp)	Genotype									
		Pe.km1	Pe.km2	Pe.kp3	Pe.kp4	Pe.kp5	Pe.mi6	Pe.mi7	Pe.mi8	Po.ep9	Po.ep10
HB9											
1	1320	0	0	0	0	0	0	1	1	1	1
2	1290	1	1	0	0	0	0	0	0	0	0
3	980	0	0	0	0	0	0	1	1	0	0
4	850	1	1	1	1	1	1	1	0	1	1
5	780	0	0	0	0	0	0	0	1	1	1
6	750	0	0	0	0	0	0	0	0	0	1
7	640	1	1	1	0	0	1	1	0	0	0
8	430	1	1	1	1	1	1	1	0	0	0
9	240	1	1	1	1	1	1	1	1	1	1
Total		5	5	4	3	3	4	6	4	4	4
HB10											
1	1250	1	1	1	1	1	1	1	0	0	0
2	1100	0	0	0	0	0	1	1	0	0	0
3	1050	0	0	0	0	0	0	0	1	0	0
4	680	1	1	1	1	1	1	1	1	1	1
5	570	0	0	0	0	0	1	0	0	0	0
6	400	0	0	0	0	0	1	1	1	1	1
Total		2	2	2	2	2	5	4	3	2	2
HB11											
1	1110	1	1	0	1	0	1	1	1	1	1
2	1040	1	1	0	0	0	1	1	1	1	1
3	970	1	1	0	0	0	0	0	1	1	0
4	650	1	1	0	0	0	1	1	0	1	0
5	480	1	1	1	1	1	1	1	1	1	1
6	290	0	0	0	0	0	0	0	1	0	0
Total		5	5	1	2	1	4	4	5	5	3
HB13											
1	1230	0	1	0	0	0	0	0	0	0	0
2	1200	0	1	0	0	0	0	0	0	0	0
3	1040	1	1	1	1	1	1	1	1	1	1
4	980	1	1	1	1	1	1	1	1	1	1
5	870	0	0	0	0	0	0	1	1	1	1
6	780	0	0	0	0	0	0	0	1	1	0
7	640	1	1	1	1	1	1	1	0	0	0
8	530	0	0	1	0	0	0	0	0	1	0
9	310	0	0	1	0	0	0	0	0	0	0
Total		3	5	5	3	3	3	4	4	5	3
HB14											
1	2130	1	1	1	1	0	0	0	0	0	0
2	1190	0	1	1	1	0	1	1	1	0	0
3	1040	0	1	1	0	0	0	0	1	0	0
4	980	1	0	0	0	0	0	0	0	0	0
5	860	1	1	1	1	0	1	1	1	1	1
6	820	0	1	0	0	0	1	1	1	0	0
7	710	1	1	1	1	0	1	1	1	1	1
8	620	1	1	1	1	0	0	1	1	1	0
9	540	1	1	1	1	1	1	1	1	1	1
10	430	1	1	1	1	1	1	1	1	1	1
11	310	1	1	1	1	1	1	1	1	1	1
12	280	1	0	0	0	0	0	0	1	1	0
13	220	0	0	0	0	0	1	1	1	1	0
14	150	1	1	1	1	1	1	1	1	1	1
Total		10	11	10	9	4	9	10	12	9	6

According to the polymorphism resulting RAPD reaction, the percentage of polymorphic bands being the lowest (25%) in case of primer opo10 and the highest is (57.14%) in case of primer oPo12. The percentage of polymorphic bands generated from ISSR reaction being the lowest (71.42%) in case of primer HB14 and the highest (100%) in case of primer HB9 as shown in Table (5).

Table (5): The range of band products, number of common and polymorphic bands produced by different primers used for studied species.

	Primer	Range of products	Total bands	Monomorphicbands	Polymorphic bands	Polymorphism %
RAPD	OPo9	320-990	11	6	5	45.45
	OPo10	340-980	12	9	3	25
	OPo11	550-980	5	3	2	40
	OPo12	470-1150	7	3	4	57.14
	OPo13	350-1050	9	6	3	33.33
ISSR	HB9	240-1320	9	-	9	100
	HB10	400-1250	6	1	5	83.33
	HB11	290-1110	6	1	5	83.33
	HB13	310-1230	9	2	7	77.77
	HB14	150-2130	14	4	10	71.42

Similarity indices between the studied accessions of *Persicaria* and *Polygonum* which collected from different localities in Egypt revealed that the lowest similarity value (0.530) was recorded between *Pe.lan1*collected from EL-Adlia EL-Nile (EL-Salaam)-Damietta Governorate and *Po.equ10* collected from Gamasa coast Dakahlya Governerate. The highest similarity value (0.805) founded between *Pe.sal8* collected from Kafr EL-Tawila (Talkha) Dakahlya Governorate and *Po.equ9* collected from Gamasa coast Dakahlya Governorate. These results were obtained by using different compiled techinques includes molecular (RAPD & ISSR) and protein, indicates the relation between these different species with different localities as shown in Table (6).

Table (6): The Spearman correlations matrix between the studied accessions of *Persicaria* and *Polygonum* in Egypt (Name of accessions see Table1).

	Similarity matrix									
plant Species	*Pe.lan 1*	*Pe.lan 2*	*Pe.lap 3*	*Pe.lap 4*	*Pe.lap 5*	*Pe.sal 6*	*Pe.sal 7*	*Pe sal8*	*Po.equ 9*	*Po.equ 10*
Pe. lan1	1									
Pe. lan2	0.753	1								
Pe. lap3	0.646	0.684	1							
Pe. lap4	0.658	0.697	0.797	1						
Pe. lap5	0.582	0.620	0.732	0.776	1					
Pe. sal6	0.622	0.639	0.638	0.693	0.726	1				
Pe. sal7	0.634	0.631	0.671	0.662	0.568	0.731	1			
Pe. sal8	0.543	0.593	0.573	0.600	0.552	0.607	0.735	1		
Po.equ9	0.605	0.584	0.619	0.610	0.598	0.635	0.707	0.805	1	
Po.equ10	0.530	0.566	0.582	0.613	0.622	0.600	0.654	0.734	0.750	1

By illustrating possiple relationships among the ten studied accessions of genus *Persicaria* and *Polygonum* based on all compiled matrix data. The investigated accessions were divided mainly into two groups at distance 0.606. The second group is further divided into two subgroups at a distance of 0.641. The first subgroup also was divided into two subgroups at 0.660. The first one includes *Pe.sal7* collected from EL-Westani Drainge canal- Dakahlya Governorate and *Pe.sal8* collected from Kafer EL-Tawila (Talkha) Dakahlya Governorate whilst the second subdivision include all accessions of *Persicaria lapathifolia* collected from different localities in Egypt as shown in Table (1). The second group includes *Pe.lan1* and *Pe.lan2* were collected from EL-Adlia EL-Nile (EL-Salaam)-Damietta Governorate and El-Motamadya village –El-Gharbya Governorate, respectively as shown in Figure 2.

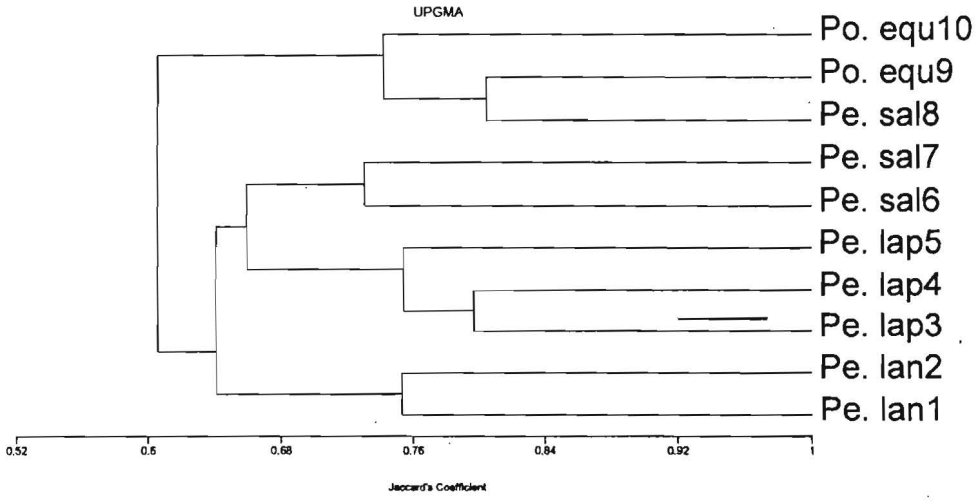

Fig (2): Phenogram showing the relationships between the studied *Persicaria* and *Polygonum* species from different accessions in Egypt using based on similarity indices using UPGMA and Jaccard's Coefficient (Names of accessions see Table1).

Conclusion

Molecular biology assays (PCR-based) using RAPD and ISSR primers beside that biochemical study all these studies revealed the presence of great variability among the genotype of *Persicaria* and *Polygonum* species and were succeeded either together or separately for identification and characterization of these four species.

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التوصيف البيوكيميائى والوراثه الجزيئية لأربعة أنواع من الفصيله الحماضية فى مصر.

ماجده إبراهيم سليمان ، ياسر أحمد الأمير، إيمان محمد فايد

قسم النبات – كلية العلوم – جامعة المنصوره- مصر.

يهدف هذا البحث إلى دراسة التباين الوراثى لأربعة أنواع من الفصيله الحماضية النامية فى مصر والنباتات قيد الدراسة هى نبات أبو ركبة(*Persicaria lanigera*)وعكريش(*Persicaria lapathifolia*) وقرضاب (*Persicaria salicifolia*) وعفراج (*Polygonum equisetiforme*).

وقد تم تجميع هذه النباتات من أماكنها الطبيعية . وقد تم إجراء دراسة بيوكيميائية تتمثل فى تعيين أنواع البروتينات باستخدام التفريد الكهربى للبروتينات (SDS-PAGE) للأنواع قيد الدراسة وتم رصد إجمالى ١٧ حزمة يتراوح وزنها الجزيئي من ١١ إلى ١٠٠ كيلو دالتون.

وقد تم تعين مدى التنوع الوراثى على المستوى الجزيئي عن طريق دراسة التكبير العشوائى متعدد الأشكال (RAPD) وتكرار التتابع الداخلى البسيط (ISSR) باستخدام خمسة بادئات لكلا منها للحمض النووىDNA وقد أوضحت النتائج تسجيل عدد٤٤ حزمة باستخدام طريقة RAPD وكذلك تسجيل عدد٤٤ حزمه باستخدام طريقة ISSR كما أظهرت النتائج المتحصل عليها بطريقة تكرار التتابع الداخلى البسيط ISSR وطريقة التكبير العشوائى متعدد الأشكال RAPD وجود تباينات وراثيه بين النباتات قيد الدراسة وان نسبة التنوع إختلفت من بادئ إلى آخر.