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GENETIC ASSESSMENT IN SOME MEDICINAL PLANTS UTILIZING MOLECULAR MARKERS

[148]

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ABSTRACT

The interest in medicinal plants is not only due to the fact that it is a source of food additives, but it is also a major source of medicines. Medical plants contain many important vital ingredients used in the treatment of many diseases. Therefore, medicinal plants are considered a safe source of medical drugs. Many medicinal plants have a significant economic importance to increase their demand. The plant families include many families, such as solanaceae family, which is one of the largest plant families with more than 3000 plant species And the plants of this family contains many important biological components and because of its importance, it was necessary to study and identify these plants by making fingerprinting, detect the molecular genetic markers for this family, study the genetic diversity of these species and determine the relationships between species of this family by using genetic techniques such as the technique of Inter simple sequence repeat (ISSR). The results obtained in the five solanaceae species (Lycium shwaii, Hyoscyamus muticus, Solanum nigrum from Northwestern coast, Solanum nigrun from Saint Katherine, and Nicotiana gluca) indicated that, 6 primers were applied. The HB15 primer which gave 12 bands, showed the highest polymorphism 58.33%, and the 49A primer, which gave 6 bands gave a lowest polymrphism 16.66% between the used plants populations, these results mean that ISSR technique is an powerful tool to make genetic diversity assessment for species.

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INTRODUCTION

Over the past decade, herbal medicine has become a topic of global importance, making an impact on both world health and international trade. Medicinal plants continue to play a central role in the health care system of large proportions of the human's population. This is particularly true in developing countries, where herbal medicine has along and uninterrupted history of use. Recognition and development of the medicinal and economic benefits of these plants are on the increase in both developing and industrialized nations. Continuous usage of herbal medicine by a large proportion of the population in the developing countries is largely due to the high cost of western pharmaceuticals and healthcare, (**Akerele, 1988 and WHO, 1998**). Family solanaceae is one of the most intriguing plant families in the world not only because it is one of the largest families in plant kingdom with more than 3,000 species, but also this family consists of some plants as *Atropa*, *Datura*, and *Hyoscyamus* that are important from medicinal point of view. This importance is due to their tropan alkaloid content (**Evans**, **1979**). Genetic fingerprinting has been accomplished traditionally through the use of isozymes, total seed protein and more recently through various types of molecular DNA markers. However, DNA-based markers provide powerful tools for discerning variations within crop germplasm and for studying evolutionary relationships. Among molecular markers, randomly amplified polymorphic DNAs (RAPDs) have been extensively used in genetic research owing to their speed and simplicity.

Assessment of genetic diversity and relationships within and among crop species and their wild relatives is essential not only for basic studies on

evolution but also for informed utilization and protection of plant genetic resources **(Rao and Hodgkin, 2002; Barcaccia, 2009; Brozynska et al 2015).**

Rajora and Mosseler (2001) found out that, a central objective of genetic resources conservation is to maintain genetic integrity and natural levels of genetic diversityand to enhance genetic diversity in population and species where it has been eroded. He further stated that genetic diversity is essential for the long term survival of the species by populations because it provides the raw material for adoption and evolution, especially when environmental conditions have changed.

MATERIALS AND METHODS

1- Materials

Plant Materials

The present study was carried out at Agriculture Research Center (ARC). Leaves of the five medicinal plants (*Lycium shawii , Solanum nigrum , Nicotiana glauca, Hyoscyamus muticus*) are collected from their nature habitats (North western coast and saint Catherin). These species were selected as a study site because they contain vital components, most important of which are alkaloids which have many medicinal uses.

2- Method

Inter Simple Sequence Repeat ISSR

Genomic DNA was extracted from 0.12 g of fresh leaf material from five randomly selected plants per accession using a Plant total DNA GPB Mini Kit (Geno Plast Biochemicals, Poland) according to the manufacturer's instructions. DNA quality and quantity were established by spectrophotometric measurements and agarose gel electrophoresis. Only samples of high quality were used for ISSR-PCR. ISSR-PCR amplifications were performed in reaction volumes of 25 µL, containing 30 ng of genomic DNA template, 0.1 U/µL Taq DNA polymerase, 4 mM MgCl2, 0.5 mM of each dNTPs, 10 µM primer, and sterile deionized water. Reactions were performed using a T100 Thermal Cycler (Bio-Rad, Poland) under the following conditions: initial denaturation at 94 °C for 5 min, followed by 40 cycles of 94 °C for 1 min, annealing at 47.1–67.2 °C (depending on the primer sequence) for 1 min, and 72 °C for 2 min. The last cycle was followed by a final extension step of 7 min at 72 °C. Thirty-nine ISSR primers (Genomed, Poland; previously reported by **Rewers and Jedrzejczyk, 2016**) were tested, out of which 28 generated stable band patterns and five primers were selected for further studies (Supporting Information, **Table (1)**. All reactions with the selected primers were repeated twice. Amplification products were separated using 1.5% (w/v) agarose gel electrophoresis. A DNA ladder of 3000 bp was used to determine the size of the fragments. The bands were visualized using GelDoc XR+ (Bio-Rad). The ISSR bands were counted using a binary scoring system that recorded the presence or absence of bands as 1 and 0, respectively. The number of monomorphic and polymorphic amplification products generated by each primer was determined. The polymorphism information content (PIC) was calculated according to **Ghislain et al (1999)**. Estimates of genetic distances were calculated according to **Nei and Li (1979)** and a dendrogram was constructed using the unweighted pair group method with arithmetic average (UPGMA) by the Treecon v. 3.1 program **(Van de Peer and De Wachter, 1994)**. Statistical support of the branches was tested with bootstrap analysis using 2000 replicates. The distance matrix was used for principal coordinate analysis (PCoA).

RESULTS AND DISCUSSION

In the present study, the genetic variability and relationships of six different natural medicinal plants (*Lycium shawii , Solanum nigrum , Nicotiana glauca ,* Hyoscyamus muticus) that collected from North western coast , and (*Lycium shawii , Solanum nigrum , Nicotiana glauca ,* Hyoscyamus muticus) that collected from Saint Catherin have been studied based on ISSR molecular markers .

1- Inter-simple sequence repeats (ISSR) analysis

Understanding the level of genetic diversity and the population genetic structure is important for medicinal plant species, because this allows the establishment of effective and efficient conservation practices and can guide choices for their genetic management. Nowadays, it is possible to use several molecular methods to analyze the genetic variability in plant species. One of them, inter-simple sequence repeat polymorphisms (ISSR), have been successfully used for genetic analysis of medicinal plants, as they require no prior

knowledge of the DNA sequence and are universally applicable as dominant markers **(Sa et al 2011)** for rapid exploratory work on new species. Furthermore, ISSRs have been demonstrated to be useful for the analysis of inter- and/or intra-specific genetic diversity in different Gentianaceae species **(Ge et al 2005; Zhang et al 2007; Yang et al 2011; Zheng et al 2011).**

Table 1. List of the primer names and their nucleotide sequences used in the study

No.	Name	Sequence
1	**44A**	5´ CTC TCT CTC TCT CTC TAC 3`
2	**49A**	5´ CAC ACA CAC ACA AG 3`
3	**49B**	5´ CAC ACA CAC ACA GG 3`
4	**98B**	5´ CAC ACA CAC ACA GT 3`
5	**HB8**	5´ GAG AGA GAG AGA GG 3`
6	**HB15**	5´ GTG GTG GTG GC 3`

In the present study, many of primers were used to deffrentiate between the five plants (*Lycium shawii, Solanum nigrum, Nicotiana glauca, Hyoscyamus muticus*) which collected from two different locations (North west coast, Saint Catherine). However, only six primers (44A-49A-49B-98B-HB8-HB15). Shown in **Table (2)** gave reproducible results and were reprted as follow:

The PCR products of primer 44A are illustrated in **Fig. (1) and Table (3)**. This primer produced three (3) bands ranging from 1500 pb to 500 pb. This primer produced two common bands in all five plants (from the all different locations) with molecular sizes 1000 and 500 pb.

The PCR products of primer 49A are illustrated in **Fig. (1) and Table (3)**. This primer produced six (6) bands ranging from 4000 pb to 500 pb. This primer produced five common bands in all five plants (from the different locations) with molecular sizes 3000 and 500 pb.

He PCR products of primer49B are illustrated in **Fig. (1) and Table (3)**. This primer produced Thirteeen (13) bands ranging from 2000 pb to 100 pb. This primer produced eight (8) common bands with molecular markers (900pb – 800pb – 700pb – 600pb – 500pb – 400pb – 300pb – and 200pb)in all five plants (from different locations) . On the other hand Nicotiana gluca has two common bands with molecular sizes with 2000 pb and 1500 pb. While *Solanum nigrum* from (North coast)has one specific band with molecular size 100 pb.

The PCR products of primer98B are illustrated in **Fig. (1) and Table (3)**. This primer produced three (3) bands ranging from 1500 pb to 500 pb. This primer produced two common bands in all five plants (from different locations) with molecular sizes 1000 pb and 500 pb.

The PCR products of primer HB8 are illustrated in **Fig. (1) and Table (3)**. This primer produced nine (9) bands ranging from 2000 pb to 500 pb. *Nicotiana Gluca* has two positive specific bands with molecular sizes 1200 pb and 700 pb , While *Lycium shawii* has one positive specific band with molecular size 500 pb.

The PCR products of primer HB15 are illustrated in **Fig. (1) and Table (3)**. This primer produced twelve (12) bands ranging from 1,500 pb to 100 pb. This primer produced four (4) common bands with molecular sizes (600 pb – 400 pb – 300 pb – 100 pb). *Solanum nigrum* from (North coast) has two positive specific bands with molecular sizes 1,500 pb and 1,200 pb , While *Hyoscyamus muticus* has one specific band with molecular size 700 pb.

Table 2. Amplified fragments obtained from the DNAs of the five plants using RAPD-PCR technique

AF of RAPD primers	Band No.	*Lycium shawii*	*Solanum nigrum* (North coast)	*Solanum nigrum* (Saint Catherine)	*Nicotiana glauca*	*Hyoscyamus muticus*	M.S Pb	M+M-
44A	1	1	0	1	0	0	1500	
	2	1	1	1	1	1	1000	
	3	1	1	1	1	1	500	
	Total	**3**	**2**	**3**	**2**	**2**		
49A	1	1	1	1	0	0	4000	
	2	1	1	1	1	1	3000	
	3	1	1	1	1	1	2000	
	4	1	1	1	1	1	1,500	
	5	1	1	1	1	1	1000	
	6	1	1	1	1	1	500	
	Total	**6**	**6**	**6**	**5**	**5**		
49B	1	0	0	0	1	0	2000	M+
	2	0	0	1	1	0	1,500	
	3	1	1	1	1	0	1,200	M-
	4	1	1	1	1	0	1,031	M-
	5	1	1	1	1	1	900	
	6	1	1	1	1	1	800	
	7	1	1	1	1	1	700	
	8	1	1	1	1	1	600	
	9	1	1	1	1	1	500	
	10	1	1	1	1	1	400	
	11	1	1	1	1	1	300	
	12	1	1	1	1	1	200	
	13	0	1	0	0	0	100	
	Total	**10**	**11**	**11**	**12**	**9**		
98B	1	1	1	0	0	1	1,500	
	2	1	1	1	1	1	1,000	
	3	1	1	1	1	1	500	
	Total	**3**	**3**	**2**	**2**	**3**		
HB8	1	1	1	0	1	1	2000	M-
	2	1	1	0	1	1	1,500	M-
	3	0	0	0	1	0	1,200	M+
	4	0	0	0	0	0	1,031	
	5	1	0	1	0	0	900	
	6	0	0	1	0	0	800	M+
	7	0	0	0	1	0	700	M+
	8	0	1	0	0	1	600	
	9	1	0	0	0	0	500	M+
	Total	**4**	**3**	**2**	**4**	**3**		
HB15	1	0	1	0	0	0	1,500	M+
	2	0	1	0	0	0	1,200	M+
	3	1	1	1	0	1	1,031	M-
	4	0	0	0	0	0	900	
	5	1	0	0	1	0	800	
	6	0	0	0	0	1	700	
	7	1	1	1	1	1	600	
	8	0	1	1	1	1	500	M-
	9	1	1	1	1	1	400	
	10	1	1	1	1	1	300	
	11	1	1	0	1	1	200	M-
	12	1	1	1	1	1	100	
	Total	**7**	**9**	**6**	**7**	**8**		

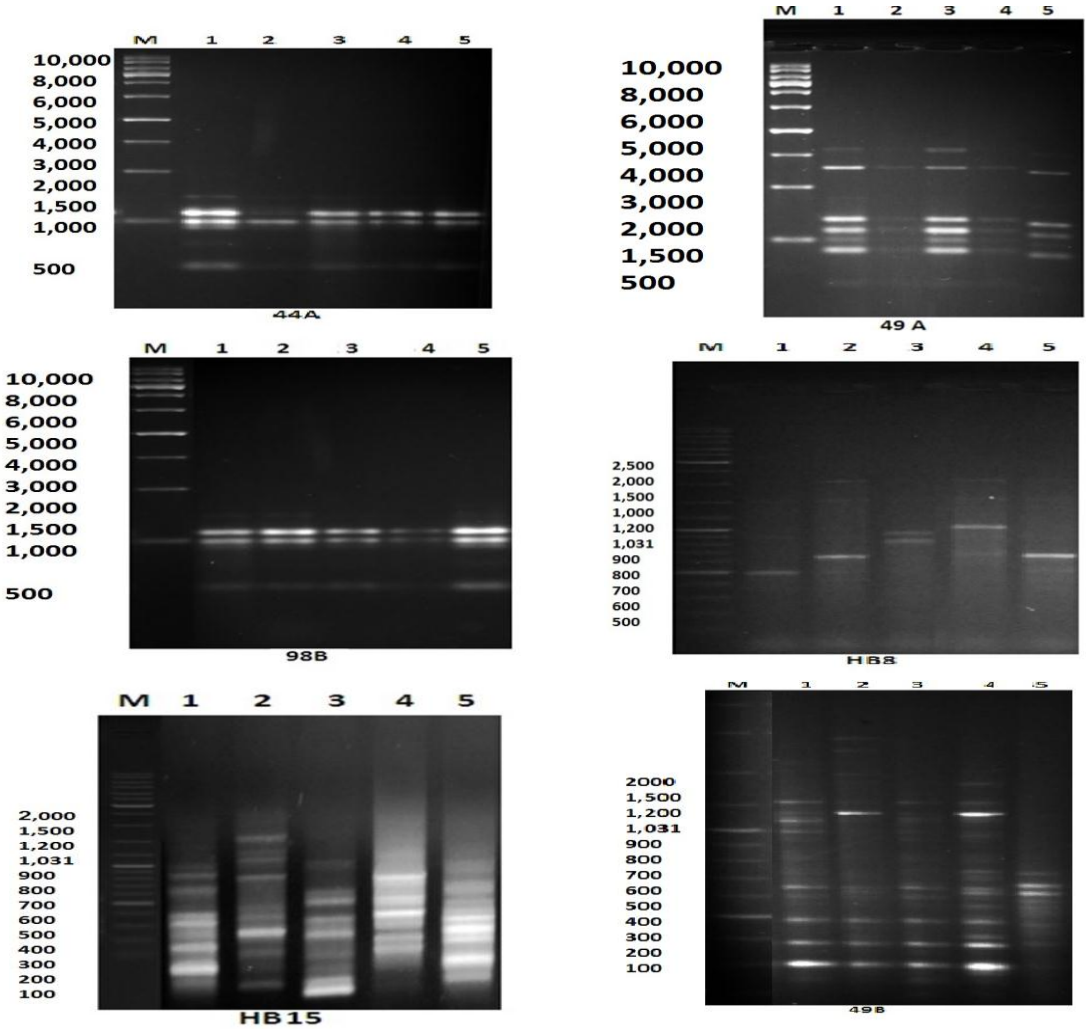

Fig. 1. ISSR banding patterns of the five species amplified with six arbitrary primers

Table 3. Amplification results of the five ISSR primers for the five solanaceae plants genotypes tested

Primers	TAF	PB	P%	Genotypes										TSM
				G1		G2		G3		G4		G5		
				AF	SM	AF	SM	AF	SM	AF	SM	AF	SM	
44A	3	1	33.33%	3	0	2	0	3	0	2	0	2	0	0
49A	6	1	16.66%	6	0	6	0	6	0	5	0	5	0	0
49B	13	5	38.46%	10	0	11	0	11	0	12	1	9	2	3
98B	3	1	33.33%	3	0	3	0	2	0	2	0	3	0	0
HB8	9	8	88.88%	4	1	3	0	2	3	4	2	3	0	6
HB15	12	7	58.33%	7	1	9	2	6	1	7	1	8		5

G1: *Lycium shwaii* G2: *Solanum nigrum* (Northwestern coast)

G3: *Solanum nigrum* (Saint Katherine) G4:*Nicotiana gluca* G5: *Hyoscyamus muticus*

TAF= Total number of amplified fragments

PB= polymorphic bands

P%= Polymorphism percentage

AF= Amplified fragments / genotype

SM= Genotype – Specific marker including either the presence or absence of a given band

TSM= Total number of specific markers

The present study aimed to survey the molecular analysis *to assess* the genetic variation of five different plant species from family solanaceae (*Lycium shwaii*, *Solamnum nigrum*, *Hyoscyamus muticus*, *Nicotiana gluca*) from North western coast and (*Lycium shwaii*, *Solanum nigrum*, *Hyoscyamus muticus*) from Saint Catherin. At the molecular level ISSR marker was applied to assesss the DNA fingerprinting between (*Lycium shwaii*, *Solanum nigrum* from north western coast, *Solanum nigrum* from Saint Catherin, *Hyoscyamus muticus*, *Nicotiana gluca*). For ISSR analysis six ISSR analysis were used to differentiate between the five plants. The results obtained from ISSR analysis resulted in 3, 6, 13, 3, 9 and 12 total bands for each species *Lycium shwaii*, *Solanum nigrum* from north western coast, *Solanum nigrum* from Saint Catherin, *Hyoscyamus muticus*, and *Nicotiana gluca*. **Elkholy et al (2011)** supported our results by using the same technique to evaluate Genetic variability in Nepeta septemcrenata populations from six locations in Saint Catherin protectorate. Also **Sarkhaill et al (2014)** and **Solyman and Alkawni (2014)** agree with this results.

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التقييم الوراثى فى بعض النباتات الطبية بإستخدام الواسمات الجزيئية

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الموجـــــز

إن الاهتمام بالنباتات الطبيه لا يرجع فقط انها مصدر للغذاء ولكنها أيضا هى مصدر رئيسى للحصول على الادويه حيث تحتوى النباتات الطبيه على العديد من المكونات الحيويه الهامه تستخدم فى علاج الكثير من الامراض وبالتالى تعتبر النباتات الطبيه مصدر امن بديلا عن العقاقير الطبيه والتى ينتج عن استخدامها اثار جانبيه عديده ، وعليه النباتات الطبيه لها أهميه إقتصاديه كبيره لزيادة الطلب عليها. وتضم العائلات النباتيه عائلات عديده منها العائله الباذنجانيه وهى واحده من أكبر العائلات النباتيه حيث تضم أكثر من 3000 نوع نباتى، وتحتوى نباتات هذه العائله على العديد من المكونات الحيويه الهامه ونظرا لاهميتها فإنه كان من الازم دراستها وتعريفها بعمل بصمه وراثيه لها، والكشف عن العلامات الجزيئيه الوراثيه لهذه الانواع ودراسة التنوع الوراقى لهذه الانواع وتحديد علاقات القرابه بين الأنواع النباتيه لهذه العائله وذلك باستخدام تقنيات وراثيه مثل تقنية التكرارات المتتابعة البينية البسيطة حيث أن النتائج المستخدمه بتقنية الـ ISSR فى ال5 أنواع النباتيه (العوسج، وعنب الديب من الساحل الشمالى الغربى، وعنب الديب من سانت كاترين، والسكران المصرى، والمصاص)، انه تم استخدام 6 بادئات، و أن البادئ HB15 والذى أعطى 12 حزمه أعلى إختلاف 58.33% وأوضح البادئ 49A والذى أعطى 6 حزم أقل إختلاف 16.66%. نتائج هذه الدراسه تعنى ان هذه التقنيه هى أداه قويه لعمل تقييم للتنوع الوراثى بين الانواع.

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