GENETIC ASSESSMENT IN SOME MEDICINAL PLANTS UTILIZING MOLECULAR MARKERS

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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 contain 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 polymorphism 16.66% between the used plants populations, these results mean that ISSR technique is a powerful tool to make genetic diversity assessment for species.

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 diversity and 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’ CTG TCT TCT TCT TCT GCC ATAC’ |
| 2   | 49A  | 5’ CAC ACA CAC ACA AG C’ |
| 3   | 49B  | 5’ CAC ACA CAC ACA GG C’ |
| 4   | 98B  | 5’ CAC ACA CAC ACA GT C’ |
| 5   | HB8  | 5’ GAG AGA GAG AGA GG C’ |
| 6   | HB15 | 5’ GTG GTG GTG GC C’ |

In the present study, many of primers were used to differentiate 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 reported 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 Thirteen (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 glauca 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 (North coast) | Solanum nigrum (Saint Catherine) | Nicotiana glauca | Hyoscyamus muticus | M.S Pb | M+M- |
|---------------------|----------|-----------------------------|---------------------------------|-----------------|-------------------|-------|------|
| 44A                 | 1        | 1                           | 1                               | 0               | 0                 | 1500  |       |
|                     | 2        | 1                           | 1                               | 1               | 1                 | 1000  |       |
|                     | 3        | 1                           | 1                               | 1               | 1                 | 500   |       |
| **Total**           | **3**    | **2**                       | **2**                           | **2**           | **2**             |       | **5**|
| 49A                 | 1        | 1                           | 1                               | 0               | 0                 | 4000  |       |
|                     | 2        | 1                           | 1                               | 1               | 1                 | 3000  |       |
|                     | 3        | 1                           | 1                               | 1               | 1                 | 2000  |       |
|                     | 4        | 1                           | 1                               | 1               | 1                 | 1,500 |       |
|                     | 5        | 1                           | 1                               | 1               | 1                 | 1000  |       |
|                     | 6        | 1                           | 1                               | 1               | 1                 | 500   |       |
| **Total**           | **6**    | **6**                       | **6**                           | **5**           | **5**             |       | **11**|
| 49B                 | 1        | 0                           | 0                               | 1               | 0                 | 2000  | M+   |
|                     | 2        | 0                           | 0                               | 1               | 0                 | 1,500 | M-   |
|                     | 3        | 1                           | 1                               | 1               | 0                 | 1,200 | M-   |
|                     | 4        | 1                           | 1                               | 1               | 0                 | 1,031 | M-   |
|                     | 5        | 1                           | 1                               | 1               | 1                 | 900   |       |
|                     | 6        | 1                           | 1                               | 1               | 1                 | 800   |       |
|                     | 7        | 1                           | 1                               | 1               | 1                 | 700   |       |
|                     | 8        | 1                           | 1                               | 1               | 1                 | 600   |       |
|                     | 9        | 1                           | 1                               | 1               | 1                 | 500   |       |
|                     | 10       | 1                           | 1                               | 1               | 1                 | 400   |       |
|                     | 11       | 1                           | 1                               | 1               | 1                 | 300   |       |
|                     | 12       | 1                           | 1                               | 1               | 1                 | 200   |       |
|                     | 13       | 0                           | 0                               | 0               | 0                 | 100   |       |
| **Total**           | **10**   | **11**                      | **11**                          | **12**          | **9**             |       | **20**|
| 98B                 | 1        | 1                           | 1                               | 0               | 1                 | 1,500 |       |
|                     | 2        | 1                           | 1                               | 1               | 1                 | 1,000 |       |
|                     | 3        | 1                           | 1                               | 1               | 1                 | 500   |       |
| **Total**           | **3**    | **3**                       | **2**                           | **2**           | **3**             |       | **8**|
| HB8                 | 1        | 1                           | 1                               | 0               | 1                 | 2000  | M-   |
|                     | 2        | 1                           | 1                               | 0               | 1                 | 1,500 | M-   |
|                     | 3        | 0                           | 0                               | 0               | 1                 | 1,200 | M+   |
|                     | 4        | 0                           | 0                               | 0               | 0                 | 1,031 | M+   |
|                     | 5        | 1                           | 0                               | 1               | 0                 | 900   |       |
|                     | 6        | 0                           | 0                               | 1               | 0                 | 800   | M+   |
|                     | 7        | 0                           | 0                               | 0               | 1                 | 700   | M+   |
|                     | 8        | 0                           | 1                               | 0               | 0                 | 600   |       |
|                     | 9        | 1                           | 0                               | 0               | 0                 | 500   | M+   |
| **Total**           | **4**    | **3**                       | **2**                           | **4**           | **3**             |       | **9**|
| HB15                | 1        | 0                           | 1                               | 0               | 0                 | 1,500 | M+   |
|                     | 2        | 0                           | 1                               | 0               | 0                 | 1,200 | M+   |
|                     | 3        | 1                           | 1                               | 0               | 1                 | 1,031 | M+   |
|                     | 4        | 0                           | 0                               | 0               | 0                 | 900   |       |
|                     | 5        | 1                           | 0                               | 0               | 1                 | 800   |       |
|                     | 6        | 0                           | 0                               | 0               | 1                 | 700   |       |
|                     | 7        | 1                           | 1                               | 1               | 1                 | 600   |       |
|                     | 8        | 0                           | 1                               | 1               | 1                 | 500   | M-   |
|                     | 9        | 1                           | 1                               | 1               | 1                 | 400   |       |
|                     | 10       | 1                           | 1                               | 1               | 1                 | 300   |       |
|                     | 11       | 1                           | 0                               | 1               | 1                 | 200   | M-   |
|                     | 12       | 1                           | 0                               | 1               | 1                 | 100   |       |
| **Total**           | **7**    | **9**                       | **6**                           | **7**           | **8**             |       | **20**|
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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%  | G1 AF | G1 SM | G2 AF | G2 SM | G3 AF | G3 SM | G4 AF | G4 SM | G5 AF | G5 SM | TSM |
|---------|-----|----|-----|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-----|
| 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 gluba  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, Solanum nigrum, Hyoscyamus muticus, Nicotiana glauca) from North western coast and (Lycium shwaii, Solanum nigrum, Hyoscyamus muticus) from Saint Catherin. At the molecular level ISSR marker was applied to assess the DNA fingerprinting between (Lycium shwaii, Solanum nigrum) from north western coast, Solanum nigrum from Saint Catherin, Hyoscyamus muticus, and Nicotiana glauca. 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 glauca. 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 الوراثي في بعض النباتات الطبية باستخدام الواسمات الجزيئية

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الكلمات الدالة: النباتات الطبية، العائلات الباذنجانية، التقييم الوراثي، تقنية تكرار التتابعات البسيطة

الموجز

إن الاهتمام بالنباتات الطبية لا يرجع فقط أنها مصدر للعقار، ولكنها أيضا هي مصدر رئيسي للحصول على الأدوية حيث تحتوي النباتات الطبية على العديد من المكونات الحيوية الهامة. تستخدم في علاج الكثير من الأمراض وبالتالي تعتبر النباتات الطبية مصدر أمن للعقارات التي تحتاج لاستخدامها أثناء العلاج. وعلي النباتات الطبية لها أهمية اقتصادية كبيرة لزيادة المبيع عليها.

وتضمن العائلات النباتية عائلات عديدة منها السوائل والباذنجانية وهو واحد من أكبر العائلات النباتية حيث تضم أكثر من 3000 نوع نباتي، وتحتوي نباتات

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