Genetic studies on *Artemisia monosperma* and *Jatropha curcas* plants by using RAPD technical

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**ABSTRACT**

In the present study, two of the Egyptian *Artemisia monosperma* and two *Jatropha curcas* genotypes were selected from Egypt, for genetics analysis by using four RAPD markers. The results indicated that the RAPD primers were able to amplify DNA fragments showing, polymorphic DNA amplification patterns among the genotypes. The data indicated that out of the total 134 bands, 57 were polymorphic (57%). The number of the observed bands ranged from two for the primer j.OPA_07, a.OPA_07 to one in the primer j.OPA_07, a.OPA_07 with an average of 2.3 across for the tested genotypes. Consequently, most of the two RAPD loci in this study were useful for the evaluation of genetic diversity between the two Egyptian *Artemisia monosperma*, Jatropha genotypes. However, it is worth mentioning, this value expresses the existence to different alleles of one or more loci of homologous chromosomes. Polymorphism data content (PIC) value of the Artemisia, Jatropha genomic RAPD indicated temperate to high level of in formativeness with average PIC value to 0.23 over all the tested RAPD loci primers, where it ranged from 0.29 by using to aOPA-07 to 0.27 in jOPA-02 for all genotypes under research by a mean to 0.23.

The genetic analysis of the three Artemisia, Jatropha based on 2 RABD markers detected 53 separate specific alleles, It is noted that, largest number patented to the specific/unique alleles were of the genotype number G2 and G3 where 53 specific alleles were patented to this genotype only.

**Key words:** RAPD, Artemisia monosperma, Jatropha curcas

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Received: 31/3/2022
Accepted: 13/4/2022
INTRODUCTION:

The genus Artemisia L., a member of the asteraceae family, comprises around 500 species of herbs growing mostly in the northern hemisphere [Bremer and Humphries ., (1993)]. It has been divided into five large sections namely: Absinthium DC, Artemisia L., Dracunculus Besser, Seriphidium Besser and Tridentatae (Rybd.) [Torrell and Vallès ., (2001)]. A general review of different systematic and evolutionary aspects of the genus, with special emphasis on cytogenetic and molecular data was given by [Vallès and McArthur., (2001)]. Over than 170 species are classified under Jatropha genus which is a widespread in the world. The species can be monoecious or dioecious, trees, shrubs rhizomatous subshrubs, or geophytes, and herbs, including some annual taxa [Kikuchi ., et al., (2011)]

folk medicine. Its seed are used in soap oil and biodiesel production. Jatropha is a diploid plant with 2n=2x=22 and a genome size is approximately 370 Mb [Carvalho., et al., (2008 )]. Jatropha genome sequences have been previously obtained by whole-genome sequencing and have been updated with newly assembled nonredundant sequences of approximately 298 Mbp from 39,277 contigs including 25,433 predicted genes [Sato et al., (2010)].

The dominant marker systems, such as AFLP, RAPD, and ISSR, among others, have the advantage of generating a large number of DNA bands, but have the disadvantage of not revealing the proportion of homozygous and heterozygous individuals[Hirakawa et al., (2012)]. The present study was conducted to achieve the following objectives:- 1-Molecular characterization analysis for the RAPD loci used to help and serve in the construction of the molecular genetic data base for Egyptian Jatropha and Artemisia. 2- Analyse the DNA fingerprinting data for identification and discrimination of some Egyptian Jatropha, Artemisia genotypes using RABD markers. 3- Study of genetic diversity level and relationships among Jatropha (Jatropha curcas L.) and Artemisia genotypes grown in Egypt, for their importance in breeding and genetic improvement programs.

MATERIALS AND METHODS:

The present study was carried out in the laboratory of biotechnology Cairo University Research Park (CURP).

Plant Materials and preparation of extracts(G.M.C.A ) :

The samples to Artemisia and Jatropha from each genotype were taken to assessment.

Genetic analysis

The present study was aiming to molecular characterization analysis for RAPD loci, analysis of the DNA fingerprinting data, study of genetic diversity level and relationships among Jatropha plant and Artemisia plant genotypes grown in Egypt. 

Methods

The total DNA of the two genotypes was extracted from frozen leaf tissue, according to CTAB method [Rogers and Bendich (1985)].
Table 1. A list of four RAPD markers included forward sequence selected references

<table>
<thead>
<tr>
<th>Loci code</th>
<th>RAPD type</th>
<th>Sequence (5→3)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>aOPA-02</td>
<td>Genomic</td>
<td>F: TGCCGAGCTG</td>
<td>Abdelfattah et al., (2012)</td>
</tr>
<tr>
<td>jOPA-02</td>
<td>Genomic</td>
<td>F: TGCCGAGCTG</td>
<td>Abdelfattah et al., (2012)</td>
</tr>
</tbody>
</table>

Amaster mix was prepared in a 2ml Eppendorf tube according to the number of PCR reactions to be performed, with an extra reaction included to compensate the loss part of the solution due to frequent pipetting. An aliquot of 23µl master mix solution was dispensed in each PCR tube (0.2ml eppendorf tube), containing 2µl of the appropriate template DNA. ; PCR reaction components are presented in Table (2).

Table 2. Components of PCR reaction.

<table>
<thead>
<tr>
<th>PCR Component</th>
<th>Amount of one PCR reaction (1X)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5X PCR buffer</td>
<td>5 µl</td>
</tr>
<tr>
<td>MgCl₂ (25 mM)</td>
<td>2.0 µl</td>
</tr>
<tr>
<td>dNTP’s mix (2 mM)</td>
<td>2.5 µl</td>
</tr>
<tr>
<td>Forward Primer (10pmoles/µl)</td>
<td>2 µl</td>
</tr>
<tr>
<td>Taq (5 U/µl)</td>
<td>0.2 µl</td>
</tr>
<tr>
<td>DNA</td>
<td>2 µl</td>
</tr>
<tr>
<td>d.dH₂O</td>
<td>9.3 µl</td>
</tr>
<tr>
<td>Total volume</td>
<td>25 µl</td>
</tr>
</tbody>
</table>

2.B.2- Molecular data analysis

Amplification products were performed with all the selected RABD primers, and only clear and reproducible bands were scored. Data was scored as one for the presence and zero for the absence of scored band of each genotype.

The genetic information was assessed for all RAPD loci using the following parameters, Observed number of alleles per locus (na), counts the number of alleles with zero allele (the alleles in case that no products were amplified in one or more genotypes), percent of polymorphic bands per RAPD locus, maximum number of alleles per genotype, average of polymorphism, the number of unique (specific) alleles per genotype, and observed heterozygosity (Ho), as direct count and it was calculated by dividing the number of heterozygous samples per locus to the total number of samples or genotypes.
The effective number of alleles (NE) was calculated for each marker according to [Heller J. (1996)] using the formula: NE = 1/∑ (E/F), where E refer to the total number of genotypes at each allele of locus i, and F the total number of alleles of the locus i in all genotypes. The heterozygosity index which also known as, polymorphism information contents (PIC) was calculated for each locus depends on number of alleles and the allele frequency from the formula: PIC = 1- ∑ pi2 where pi is the frequency of each allele. If PIC value calculated in this way, it is similar to the expression 'gene diversity' as described by [Botstein D. et al., (1980)]. Discriminating power for each locus (PD) was calculated as previous formula, but the allele frequency was replaced by the fragment or genotype frequency, according to [Kloosterman A. D., Budowlew B. and Daselaar P. (1993)]. Also, the heterozygosity level within each genotype was calculated. The genetic similarity coefficient (GS) between samples was estimated by the Dice coefficient.

Dice formula: GS (ij) = 2a/ (2a+b+c)  
Where GS (ij) is the measure of the genetic similarity between individuals i and j, (a) is the number of bands shared by i and j, (b) is the number of bands present in (i) and (c) is the number of bands absent in i and present in j. The similarity matrix was used to construct the cluster analysis. The cluster analysis was used to organize the observed data into meaningful structures, that is, to develop taxonomies. At the first step, when each sample represents its own cluster, the distances between these samples are defined by the chosen distance measure (Dice coefficient). However, once several genotypes or samples have been linked together, the distance between two clusters is calculated as the average distance between all pairs of genotypes in the two different clusters.

The computations were performed with the programs, GENEPOP version 1.31, SPSS version 16 [Rogers and Bendich (1985)]. In addition, Odds ratio data were generated by using MEDCALC™ statistical software.

**Statistical analysis**

All data were tabulated, calculated and statistically analyzed using the computer program SPSS software for windows version 22.0 (Statistical Package for Social Science, Armonk, NY: IBM Corp). Descriptive statistics was calculated in the form of Mean ± Standard deviation (SD).

### 3. RESULTS AND DISCUSSION:

**Genetic analysis**

**Molecular characterization and RAPD markers informative**

Jatropha (Jatropha curcas L.), Artemisia monosperma is a diploid species with 2n=2x=22 chromosomes and it has a shrub nature, originated from Central America and spread to Africa and Asia by Portuguese traders during 18th century [Raymond and Rouset (1995)]. Jatropha belongsto the family Euphorbiaceae and includes about 170 species. The plant is monoecious and the flowers are generally unisexual with occasional hermaphrodite flowers. Although, it has been shown that J. curcas can be geitonogamic (autogamy). Jatropha seed is high in oil content and considered promising as an alternative renewable and ecofriendly energy source, especially for biodiesel.
Table 3. Various parameters and molecular characterization of the PCR products for Two markers (RAPD) to Jatropha sample and Artemisia plant

<table>
<thead>
<tr>
<th>No</th>
<th>loci code</th>
<th>Total alleles (Na)</th>
<th>Polymorphic</th>
<th>Maximum number of alleles/genotype</th>
<th>Matching of fingerprint</th>
<th>NE&lt;sup&gt;a&lt;/sup&gt;</th>
<th>PIC&lt;sup&gt;b&lt;/sup&gt;</th>
<th>DP&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>aOPA-02</td>
<td>26</td>
<td>13</td>
<td>2</td>
<td>.0013</td>
<td>13</td>
<td>0.19</td>
<td>0.81</td>
</tr>
<tr>
<td>2</td>
<td>aOPA-07</td>
<td>42</td>
<td>17</td>
<td>2.4</td>
<td>.17</td>
<td>17</td>
<td>0.29</td>
<td>0.71</td>
</tr>
<tr>
<td>3</td>
<td>jOPA-02</td>
<td>38</td>
<td>16</td>
<td>2.3</td>
<td>.16</td>
<td>16</td>
<td>0.27</td>
<td>0.73</td>
</tr>
<tr>
<td>4</td>
<td>jOPA-07</td>
<td>28</td>
<td>11</td>
<td>2.5</td>
<td>.11</td>
<td>11</td>
<td>0.19</td>
<td>0.81</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>134</td>
<td>57</td>
<td>9.2</td>
<td>.44</td>
<td>57</td>
<td>0.94</td>
<td>3.06</td>
</tr>
<tr>
<td></td>
<td>% polymorphism</td>
<td>0.57</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mean</td>
<td>33.5</td>
<td>14.25</td>
<td>2.3</td>
<td>.11</td>
<td>14.25</td>
<td>0.23</td>
<td>0.76</td>
</tr>
</tbody>
</table>

<sup>a</sup>Effective number of alleles

<sup>b</sup>The polymorphism information content or expected heterozygositiy, and it is calculated according to Nei (1973) and reflect the ability of a marker for detecting polymorphism between the genotypes, depending on the numbers of detectable alleles and their frequency.

<sup>c</sup>The probability of discriminating between two genotypes or more with every locus. It is calculated as 1-P (P = probability of matching fingerprints).

It is worth mentioning, that most of RAPD loci in the present study produced amplicon size within the expected range., especially these genotypes have originated in a limited geographical area. Additionally, nature of the self-pollination in *Artemisia monosperma*, Jatropha has the greatest impact. Polymorphism Information Content (PIC) or gene diversity, which express of the appreciation of the discriminatory power of each RAPD locus. It is calculated depending on both the number of alleles per locus and their frequency distribution within the genotypes. This was used to evaluate their informativeness level and accordingly defined into high (PIC > 0.5), moderate (0.5 > PIC > 0.25), and low (PIC < 0.25) categories. This result was comparable to those obtained by [Tanya et al., (2011)], Most RAPD loci in this study showed PIC values more than 0.29; hence, it can be described as moderately informative to study genetic diversity.
Fig. 1. locus A.OPA_02
Fig. 2. locus A.OPA_07
Fig. 3. locus J.OPA_02
Fig. 4. locus J.OPA_07

Fig. (1-4): RAPD profiles as detected with loci jOPA_2, jOPA_7, aOPA_02 and aOPB_07. Whereas, M. refers to DNA ladder, lane 1 to 2 refers to Egyptian Jatropha and Artemisia plant genotypes in the present study.

Fig. (1-4): RAPD profiles as detected with loci jOPA_2, jOPA_7, aOPA_02 and aOPA_07. Whereas, M. refers to DNA ladder, lane 1 to 2 refers to Egyptian Jatropha and Artemisia plant genotypes in the present study.
According to the results, there was an average of 14.2 amplified bands per primer and 53% polymorphism, indicating a marked genetic variation in the examined populations. Also [Abdelfattah B. et al., (2012)] obtained in their studies using the analysis and using in their studies the analysis of morphological variation and molecular polymorphism as revealed by random amplified polymorphic DNA.

Table 4. Number of different genotypes in each genotype.

<table>
<thead>
<tr>
<th>Locus</th>
<th>Polymorphic band</th>
<th>Total No. bands</th>
<th>% Polymorphism</th>
<th>Size range (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>a.OPA-02</td>
<td>13</td>
<td>26</td>
<td>36%</td>
<td>100-1000</td>
</tr>
<tr>
<td>a.OPA-07</td>
<td>17</td>
<td>42</td>
<td>40.47%</td>
<td>100-900</td>
</tr>
<tr>
<td>j.OPA-02</td>
<td>16</td>
<td>38</td>
<td>42.1%</td>
<td>100-10000</td>
</tr>
<tr>
<td>j.OPA-7</td>
<td>11</td>
<td>28</td>
<td>39.2%</td>
<td>100-900</td>
</tr>
</tbody>
</table>

(OPA-02, OPA-05, OPA-07, OPA-08 and OPA-09) of ten Artemisia plants confirmed the variance between A. monosperma and A. judaica as two distinct species. They showed wider variations among A. judaica populations compared to those of A. monosperma populations. Karyotype analysis revealed that all A. monosperma populations are tetraploid with \(2n=36\) and a basic number of \(x=9\), while all A. Judaica samples are diploid with \(2n=16\) and \(x=8\). Like most other species to Artemisia both species have uniform karyotype but the chromosomes of A. monosperma are generally shorter and three populations to this species have a B chromosome.
REFERENCES:


RAPD دراسات وراثية على نباتات الارتميسيا والجاتروا فا باستخدام تكنيك ال

أحمد ناشت محمد عبده

ملخص

تعد هذه الدراسة، تم اختيار اثنين من التراكيب الوراثية المصرية لنبات الارتميسيا واثنين من التراكيب الوراثية المصرية لنبات الجاتروفا في مصر، وتم التشخيص الوراثي باستخدام 4 من RAPD البادئة، وتكنيك RAPD أظهرت النتائج أن بانوات الفصائل RAPD الحمض النووي التي تظهر أنماط تضخيم الحمض النووي متعدد الأشكال بين التراكيب الوراثية، وأشارت النتائج إلى أنه من إجمالي 134 혈زمه، كان 57 منها متعدد الأشكال (57%) و تراوح عدد الأحماض المرصودة من اثنين بالنسبة إلى البوادي 0.7 إلى 7.0.

توصية

عندما التحقک RAPD بأستخدام البطاریة الجاتروفا، كان 2.3 bueno مستواياً أظهرت في النتائج أيضاً وجود حد من الأليلات لكل موقع، والتي قد تكون بسبب طبيعة تضخم في تكنيك RAPD في المناطق المتضخمة تفضل الحمض النووي، وبالتالي كانت مفيدة لتقييم التنوع الوراثي بين التراكيب الوراثية المصرية لنباتات الارتميسيا والجاتروفا، ومع ذلك، تعد النتائج تشير عن وجود أليلات مختلفة لموقع وراثي واحد أو أكثر في الكروموسومات القريبة. وكما أشارت قيمة محتوى بيانات تعدد الأشكال (PIC) إلى نبات الارتميسيا بينما في نبات الفراخ فاستخدام تقنية ال RAPD حصل درجة معتدلة إلى مستوى عال من التنسيق بمتوسط قيمة aOPA-07 و المختبرة على 0.29 باستخدام RAPD المختبرة، حيث تراحت من 0.27 إلى 0.23 لجميع الوراثيات الوراثية في البحث بمتوسط 0.23، التحليل الجيني لثلاثة أرتميسيا مومسبيرو، جاتروفا على أساس 2 من بانوات ال RAPD Kشف عن G3 محددة مفصلة، ويلاحظ أن عدد مستواي لدراسة الاختيار للأليلات المحددة / الفريدة كانت من النوع الوراثي رقم G2، حيث تم تسجيل براءة اختراع لـ 53 أليلًا محدداً لهذا النمط الجيني فقط.