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Genetic studies on artemisia (*Artemisia monosperma*) and Jatropha (*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 0.27in 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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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

Loci code	RAPD type	Sequence (5→3)	Reference
aOPA-02	Genomic	F: TGCCGAGCTG	Abdelfattah *et al.*, (2012)
aOPA-07	Genomic	F: GAAACGGGTG	Manal *et al.*, (2018)
jOPA-02	Genomic	F: TGCCGAGCTG	Abdelfattah *et al.*, (2012)
jOPA-07	Genomic	F: GAAACGGGTG	Manal *et al.*, (2018)

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.

PCR Component	Amount of one PCR reaction (1X)
5X PCR buffer	5 µl
$MgCl_2$ (25 mM)	2.0 µl
dNTP's mix (2 mM)	2.5 µl
Forward Primer (10pmoles/µl)	2 µl
Taq (5 U/µl)	0.2 µl
DNA	2 µl
$d.dH_2O$	9.3 µl
Total volume	25 µl

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)2, 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., Budowlw 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^{TM} 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 byPortuguese 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 oilcontentand 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

No	loci code	Total alleles (Na)	Polymorphic	Maximum number of alleles/ genotype	Matching of finger print	NE[a]	PIC[b]	DP[c]
1	**aOPA-02**	26	13	2	.0013	13	0.19	0.81
2	**aOPA-07**	42	17	2.4	.17	17	0.29	0.71
3	**jOPA-02**	38	16	2.3	.16	16	0.27	0.73
4	**jOPA-07**	28	11	2.5	.11	11	0.19	0.81
	Total	134	57	9.2	.44	57	.94	3.O6
	% polymorphism	0.57						
	Mean	**33.5**	**14.25**	**2.3**	**.11**	**14.25**	**0.23**	**0.76**

[a]Effective number of alleles
[b]The polymorphism information content or expected heterozygosity, 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.
[c]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 inform ativeness 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.

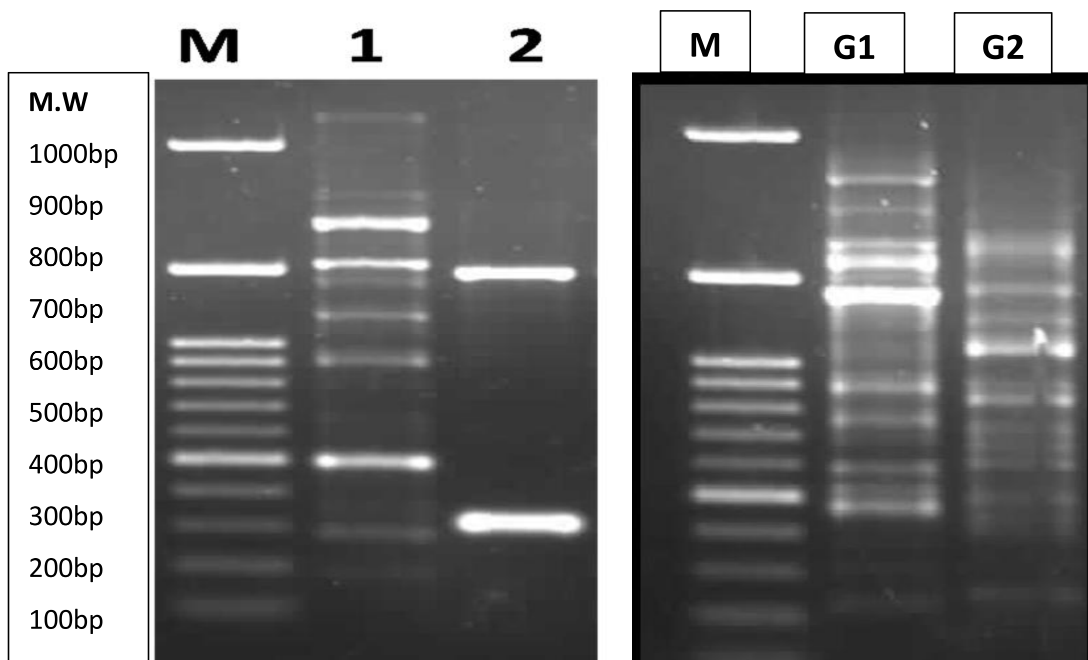

Fig. 1 . locus A.OPA_02 **Fig. 2. locus A.OPA_07**

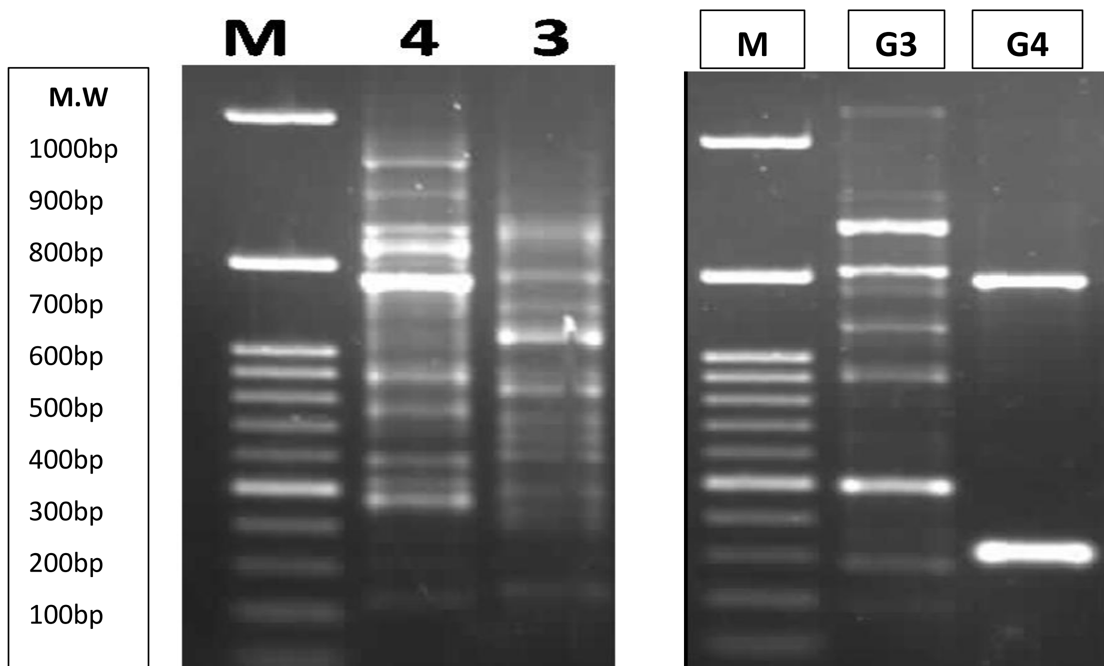

Fig. 3. locus J.OPA_02 **Fig. 4. locus J.OPA_07**

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 j.OPA_2, j.OPA_7, a.OPA_02 and a.OPA_07 Whereas, M. refers to DNA ladder, lane 1 to 2 refers to Egyptian Jatropha and Artemisia plant genotypes in the present study.

the results that there is According to the results, there 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.

Locus	Polymorphic band	Total No. bands	%Polymor phyism	Size range (bp)
a.OPA-02	**13**	**26**	**36%**	**100 -1000**
a.OPA-07	**17**	**42**	**40.47%**	**100-900**
j.OPA-02	**16**	**38**	**42.1%**	**100-10000**
j.OPA-7	**11**	**28**	**39.2%**	**100-900**

(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.

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الملخص

دراسات وراثية على نباتات الارتيمسيا والجاتروفا باستخدام تكنيك ال RAPD

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في هذه الدراسة ، تم اختيار اثنين من التراكيب الوراثية المصرية لنبات الارتيمسيا و اثنين من التراكيب الوراثية المصرية لنبات الجاتروفا فى مصر ، وتم التحليل الوراثي باستخدام 4 من البادئات وتكنيك RAPD .أشارت النتائج إلى أن بادئات RAPD كانت قادرة على تضخيم شظايا الحمض النووي التي تظهر أنماط تضخيم الحمض النووي متعدد الأشكال بين التراكيب الوراثية و أشارت النتائج إلى أنه من إجمالي 134حزمه ، كان 57 منها متعدد الأشكال (57٪) و تراوح عددالحزم المرصودة من اثنين بالنسبة إلى البوادى j.OPA-07، a.OPA-07 إلى واحد في التمهيدي j.OPA-07، j.OPA-07 بمتوسط 2.3 عبر التراكيب الوراثية المختبرة و أظهرت النتائج أيضًا وجود عدد مناسب من الأليلات لكل موقع ، والتي قد تكون بسبب طبيعة تضخيم فى تكنيكRAPD في المناطق المنسوخة لتسلسل الحمض النووي. وبالتالي كانت مفيدة لتقييم التنوع الوراثي بين التراكيب الوراثية المصرية لنباتات الارتيمسيا والجاتروفا. ومع ذلك ، تجدر الإشارة إلى أن هذه النتائج تعبر عن وجود أليلات مختلفة لموقع وراثى واحد أو أكثر فى الكروموسومات القرينة. وكما أشارت قيمة محتوى بيانات تعدد الأشكال (PIC) إلى *نبات الارتيمسيا بينما فى* نبات الجاتروفا *بأستخدام تقنية ال RAPD حقق* درجة معتدلة إلى مستوى عالٍ من التنسيق بمتوسط قيمة PIC من 0.23 على جميع بادئات مواقع ال RAPD المختبرة ، حيث تراوحت من 0.29 باستخدام aOPA-07 إلى 0.27 في jOPA-02 لجميع الأنماط الوراثية قيد البحث بمتوسط 0.23 ، التحليل الجيني لثلاثة أرتيميسيا مومسبيرما ، جاتروفا على أساس 2 من بادئات ال RAPD كشف عن 53أليل محددة منفصلة ، ويلاحظ أن أكبر عدد مسجل لبراءة اختراع للأليلات المحددة / الفريدة كانت من النوع الوراثي رقم G2، G3 حيث تم تسجيل براءة اختراع لـ 53 أليلاً محددًا لهذا النمط الجيني فقط..