

DETERMINATION OF GENETIC DIFFERENCES BETWEEN *Artemisia monosperma* PLANTS BY USING GENETIC FINGERPRINTING.

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By

A. N. M. El-Atla and *Warda Y. Ali

*Environment and Arid Land Agriculture Division, Desert Research Center,
El-Matareya, Cairo, Egypt*

ABSTRACT

In the present study, three of the Egyptian *Artemisia monosperma* genotypes were selected from Bir al-Abd city in North Sinai, Egypt, for genetics analysis by using 5 of inter simple sequence repeat (ISSR) markers. The obtained results indicated that the ISSR primers were able to amplify DNA fragments showing, polymorphic DNA amplification patterns among the genotypes. The data indicated that out of the total 120 bands, 64 were polymorphic (64%). The number of the observed bands ranged from two for the primer UBC849 to one in the primer UBC811 with an average of 2.4 across for the tested genotypes. The data also showed the presence of reasonable number of alleles for every locus in the present study, which may be due of the nature to amplified ISSR loci that most to them are located to the expressed sequence tags (EST) in the transcribed regions of the genomic DNA sequences. UBC849 with average value to 7. Consequently, most of the five ISSR loci in this study were useful for the evaluation of genetic diversity between the three Egyptian *Artemisia monosperma* 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 monosperma* genomic ISSR indicated temperate to high level of informativeness with average PIC value to 0.42 over all the tested ISSR loci primers, where it ranged from 0.07 by using to UBC849 to 0.42 in UBC811 for all genotypes under research by a mean to 0.18. Also, this study signalized a low heterozygosity level of the genotypes under research, so that for the variation between the number of alleles in each locus and number of effective alleles, so that to the existences to exclusive/specific alleles to the genotypes. The genetic analysis of the three *Artemisia monosperma* based on 5 ISSR markers detected 64 separate specific alleles, and also, that the three genotypes (G.1, G.2 and G.3) had alleles were unrivaled. So these five genotypes did not give conformable DNA-fingerprints. It is noted that, largest number patented to the specific/unique alleles were of the genotype number 3, where 30 specific alleles were patented to this genotype only.

Key words : FAA, *Artemisia monosperma*, ISSR, DNA, Fatty acids .

1. INTRODUCTION

The genus *Artemisia* L., Belongs to the Asteraceae family, compris in around 500 types of herbs growing mostly on beaches of North Africa and parts of Asia (Faten, *et al.*,2018). It has been classified into three sections: Absinthium DC, *Artemisia* L., *Dracunculus Besser*, *Seriphidium Besser* and *Tridentatae (Rybd.)*. A general review of various systematic and evolutionary aspects of the genus, with a high light on cytogenetic and molecular database, and, although that of the current in fragerenic classification does not of natural groups, and there is still without agreement

about the global treatment of the genus despite the bitter taste of *Artemisia* a plant, some insects and rodents use it as an analgesic and food (Mohammed *et al.*,2018). The plant *Artemisia* is used in desert areas as a source of wood in setting fire for cooking, as an alternative to fossil fuels and also as a material to feed animals, and it is included in the pharmaceutical industries. These, in a real and serious way, given that the *Artemisia monosperma* plant is tolerant to drought and grows naturally in poor and extremely dry lands, and what has been discovered of its importance in the biofuel industry, requires a plan to convert it into a

planted plant (Haouari and Ferchichi 2008). The sustainable conservation of the threatened plants requires evaluating the genetic diversity of different populations in different habitats to elucidate the genetic differences between related species and populations of each species (Viruel *et al.*, 2005). Sustainable conservation of natural plant species requires comprehensive knowledge of their morphological variation, karyological features and molecular genetic information that will be useful in selecting superior populations for conservation. Accordingly, the present study was conducted to achieve the following

Objectives

- 1-Molecular analysis using ISSR markers to help in the construction of the molecular genetic data based for Egyptian *Artemisia monosperma*.
- 2- Analysis of the DNA fingerprinting data for identification and discrimination of some Egyptian *Artemisia monosperma* genotypes using ISSR markers.
- 3- Measuring the concentration of fatty acids in *Artemisia monosperma* samples.

2. MATERIALS AND METHODS

The present study was carried out in the laboratory of Biotechnology Cairo University Research Park (CURP). The study aimed to examine the molecular characterization analysis for ISSR loci, and analysis of the DNA fingerprinting data, study of genetic diversity level and relationships among some *Artemisia monosperma* genotypes grown in North Sinai, Egypt, and measuring the concentration of fatty acids in *Artemisia* samples.

2. A. Molecular analysis

2. A.1. Plant Materials

Three genotypes were collected from individual plant selection program based on the vigorous growth parameters with some morphological differences. Three Egyptian genotypes of *Artemisia monosperma* were used and referred to in this study by G1 to G3. The young leaves were collected from three plants per genotype and subjected to molecular analysis.

2.A.2. Methods

2.A.2.1. Extraction and purification of Genomic DNA

The total DNA of the three genotypes was extracted from frozen leaf tissue, using the CTAB method, with slight modifications.

2.A.2.2. PCR reaction mixture

PCR amplification was carried out in 25 μ l

reaction mixture composed of 2 μ l genomic DNA (30 ng/ μ l) as a template, 10 pmoles primer, 0.2 mM of dNTP's mix (ABgene, Surrey, UK), 5X PCR buffer, 2m M MgCl₂, and 1 unit *Taq* DNA polymerase (5U/1 μ l).

PCR amplification

These five ISSR primers were synthesized by metabion international AG, Martinsried /Deutschland. PCR amplification was performed in a thermos cycler (Eppendorf Master Cycler Gradient Eppendorf, Hamburg, Germany) programmed to fulfill 35 cycles after an initial denaturation cycle for 5 min at 94°C. Each cycle consisted of a denaturation step at 94°C for 45s, an annealing step at 46°C- 56°C for 50s, and an elongation step at 72°C for 60s. The primer extension segment was extended to 7min at 72°C in the final cycle. Subsequently followed by 10 cycles of denaturation for 30 s at 94°C, annealing for 45 s at 53°C, extension for 45 s at 72°C followed by final extension for 12 min at 72°C. Every reaction was repeated two times to guarantee the reproducibility of the results.

Detection of ISSR products

A DNA molecular weight marker (100 bp or 1 kb DNA ladder, Generuler) was used as DNA size markers. After the electrophoretic run, the gels were visualized under gel documentation model (Gel-Doc 2000 with Diversity Database software Ver. 2.1, Bio-Rad Laboratories, Hercules, California, USA) for gel analysis.

Data analysis

The genetic information was assessed for all ISSR 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 ISSR locus, maximum number of bands 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 the formula: $NE = 1/\sum (E/F)^2$, where E refers 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: $PIC = 1 - \sum p_i^2$ where p_i is the

Table (1): A list of five ISSR markers, selected references and range of annealing temperature.

Loci code	ISSR Loci	Sequence (5-3)	Genbank Accessions or References	Ta	Repeat motif
UBC811	EST	GAGAGAGAGAGA GAGAC	KY399971	51.78	(GA)8
UBC818	EST	CACACACACACA CAC AG	KC864751	51.78	(CA)8
UPC 834	EST	GAGAGAGAGAGAGAGAGAT	Mona S. W. and Najat A. B.,(2014)	54.87	(GA)10
UPC849	EST	GAGAGAGAGAGAGAGAT	Mona S. W. and Najat A. B.,(2014)	47.40	(GA)8
UPC845	EST	CTCTCTCTCTG	Rana T. S.,(2013)	49	(CT)6

Ta refers to temperature of annealing

A master mix was prepared in a 2 ml 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 (0.2 ml eppendorf tube), containing 2 µl of the appropriate template DNA

frequency of each allele. If PIC value calculated in this way, it is similar to the expression 'gene diversity'. Discriminating power for each locus (PD) was calculated as previous formula, but the allele frequency was replaced by the fragment or genotype frequency. Also, the heterozygosity level within each genotype was calculated. The genetic similarity coefficient (GS) between samples

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.

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), (Table (1)).

3. RESULTS AND DISCUSSION

Artemisia monosperma is a multipurpose plant and recognized as asource energy for people living in the desert. Also it is used in veterinary purposes and therapy of many human diseases as traditional medicine in African and Asian countries. Moreover, there is a shortage of the genetic research and studies on the spread of *Artemisia monosperma* in Egypt in terms genetic diversity which benefits in breeding genetic improvement and programs and of *Artemisia monosperma*.

3.1. Molecular markers informative (Fig .1:5)

The plant is monoecious and the flowers are generally unisexual with occasional hermaphrodite flowers, although, it has been shown that *Artemisia monosperma* can be geitonogamic (autogamy). It is worth

mentioning that the *Artemisia monosperma* grading in Egypt and used in the present study belongs to Wild *Artemisia monosperma*. Currently, the only way to identify the high and low PE is the vegetative parts, by the vegetative parts analysis with high performance liquid chromatography (HPLC). *Artemisia monosperma* breeders are looking for a cheaper and quicker way to distinguish them such as through molecular markers, provided that the location of gene (s) controlling high PE content. In this study, three Egyptian *Artemisia monosperma* genotypes were used to analyse the molecular characterization and informative ISSR markers. Primers pairs were screened to reveal the polymorphisms in each of the three genotypes. But only Five ISSR primers were able of amplify bands and selected for our analysis of their numerous and polymorphic DNA amplification modality among the genotypes (Table 2) and (Fig. 2). The five ISSR loci considered, 8 of them produced only one or two bands per genotype and described as a single locus (Viruel *et al.*, 2005). However, the other 5 loci (UBC811., UBC818., UPC 834., UPC849 and UPC845) produced more bands (two-three bands with some genotypes) than expected according to the diploid texture of this specie (if used ISSR markers). Analysis to the The plant is monoecious and the flowers are generally unisexual with occasional hermaphrodite flowers, although, it has been shown that *Artemisia monosperma* can be geitonogamic (autogamy). It is worth mentioning that the *Artemisia monosperma* grading in Egypt and used in the present study belongs to Wild *Artemisia monosperma*. Currently, the only way to identify the high and low PE is the vegetative parts, by the vegetative parts analysis with high performance liquid chromatography (HPLC). *Artemisia monosperma* breeders are looking for a cheaper

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terraces. participating in, asource to genetic variation between the different genetic sites with *Artemisia monosperma* strains. The two loci in this search produced a few number to alleles and Tanya *et al.* (2013). In the same context. Vischi M., *et al.* (2017) reported very limited number of alleles ranged from 2 to 3 alleles per locus using 120 ISSR loci, Whereas, the estimated number to ISSR alleles depends on the number to samples and the genetic variability of the samples in addition to the number of ISSRs primers used and their genome coverage. Also, the difference in the number to amplified alleles in any ISSR locus is a result of the veration of rates for mutation to that locus .This moderate percentage to polymorphism may also have been influenced by the number to repeated nucleotides, whereas the ISSR markers in case to few number of repeated nucleotides (2 to 3 bp) are less polymorphic than of the case of a high number to repeated nucleotides as reported by Gadaleta *et al.* (2007). Because most ISSR loci in the present search consist of repeated dinucleotide, hence the percentages to polymorphism between the studied genotypes were between medium slightly above with the two ISSR loci.

Data in Table (3) that among seven natural local populations of the aromatic shrubby herb *Artemisia judaica* L. from different sites in South Sinai, Egypt ,by 10 selected RAPD primers, the results that there is an average of 8.7 amplified bands per primer and 57.47 % polymorphism, indicating a marked genetic variation in the examined populations.

Table(2):Various parameters and molecular characterization of the PCR products for three microsatellite markers (ISSR) in Three Egyptian *Artemisia monosperma* genotypes:

No	ISSR loci code	Total alleles(Na)	Polymorphic	Maximum number of alleles/Genotype	NE ^a	HO ^b	PIC ^c	DP ^d
1	UBC811	12	6	2	6	1.2	0.15	0.94
2	UBC818	26	14	6.5	14	2.8	0.33	0.40
3	UPC 834	23	15	1.5	15	3	0.30	0.85
4	UPC849	35	15	2.3	15	3	0.42	0.85
5	UPC845	23	14	1.6	14	2.8	0.30	0.86
	Total	120	64					
	%polymorphism	0.64						
	Mean	23.8	12.8	2.7	12.8	2.56	4.72	3.86

^aEffective number of alleles

^bDirect count heterozygosity or observed heterozygosity is calculated according to Nei (1973), 0.00 indicates that the locus is homozygous over all the studied genotypes.

^cThe polymorphism information content or expected heterozygosity, reflect the ability of a marker for detecting polymorphism between the genotypes, depending on the numbers of detectable alleles and their frequency.

^dThe probability of discriminating between two genotypes or more with every locus. It is calculated as 1-P (P = probability of matching fingerprints).

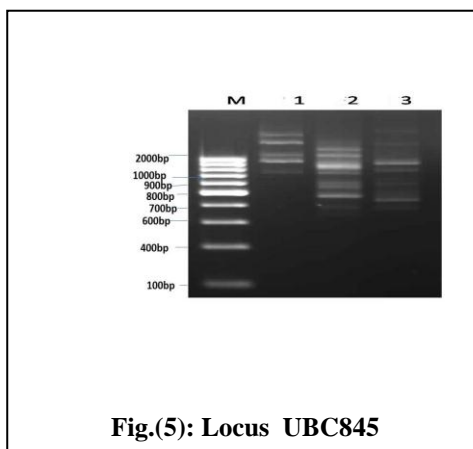
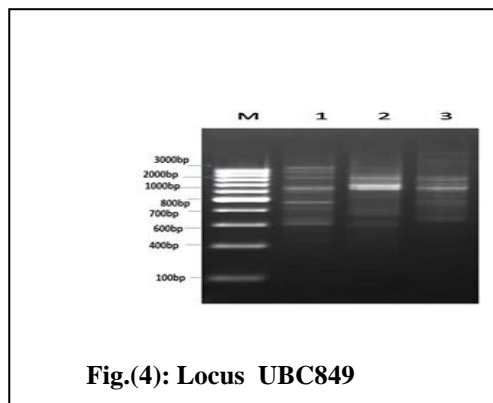
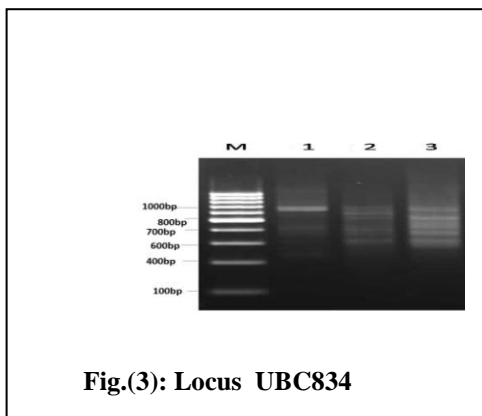
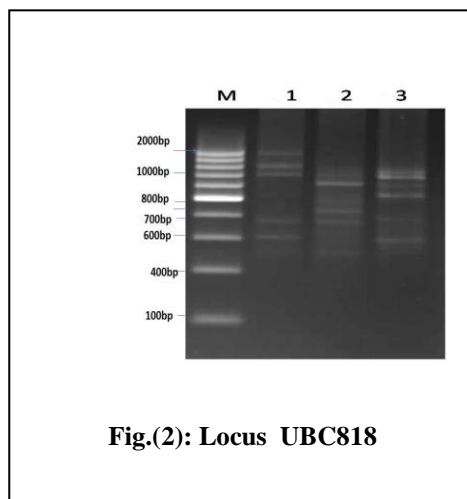
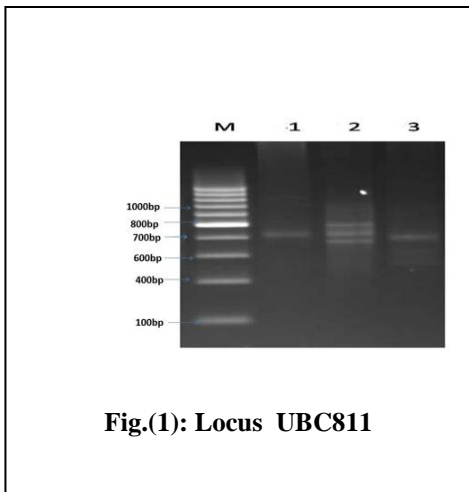
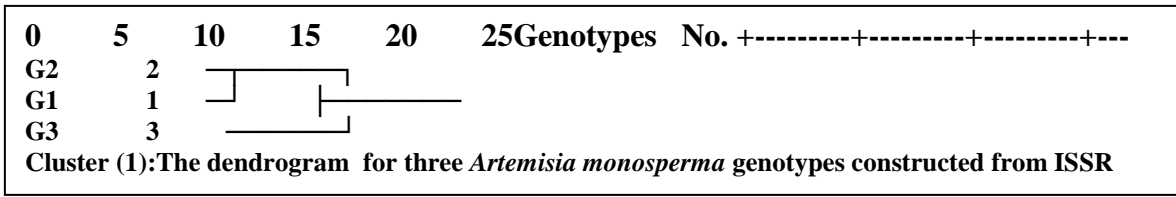


Table (3): number of different genotypes in each genotype.

Locus	Polymorphic band	Total no. bands	%Polymorphysim	Average heterozgostity	Size range (bp)
UBC811	6	12	50%	0.42	100 -700
UBC818	16	30	53%	0.16	100-1000
UBC834	14	23	70%	0.14	100-700
UBC849	19	30	63%	0.07	100-1000
UBC845	20	25	80%	0.13	100-1000

It is worth mentioning, that most of the ISSR loci in the present study produced amplicon size within the expected range. The present are in results harmony with similar analysis by Contrary, reported by Bodian *et al.* (2012). Especially these genotypes have originated in a limited geographical area. Additionally, nature of the self-pollination in *Artemisia monosperma* has the greatest impact. Polymorphism Information Content (PIC) or gene diversity, which express of the appreciation of the discriminatory power of each ISSR 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 ISSR loci in this study showed PIC values more than 0.25; hence, it can be described as moderately informative to study genetic diversity. Two EST-SSR markers, *i.e.*, UBC811., UBC818., UPC 834., UPC849 and UPC845 were most importantly showing a high degree of PIC (more than 50 %).

While Khaled and Abdelrezak, (2019) examined the genetic variability to 18 individuals of *Artemisia herba-alba* from 3 diverse elevations of Green Mountain to Libya by using sequence related amplified polymorphism (SRAP) and random amplified polymorphic DNA method, so that the results showed distinctive SRAP and RAPD banding pattern in this species scoring a total of 17 SRAP and 61 RAPD bands including 94.12 % and 100 % polymorphic with amplicon size ranging from 50 to 1600 bp and 150 to 1800 bp, respectively, and also that the Phylogenetic relationship between the genotypes was able to divide the samples into two main groups with genetic similarity value ranged from 41 to 84 %. The data obtained in such studies are very important information that may help estimate and

distribute the genetic structure contribution in conservation strategies (Ohsawa and Ide 2008). In our results the research examined a lot of genetic elevations to *Artemisia* plants under test, and its education with to Abdelfattah *et al.*,(2012) the analysis of morphological variation and molecular polymorphism as revealed by random amplified polymorphic DNA (RAPD) of ten populations of *Artemisia monosperma* and *Artemisia judaica* confirmed the differentiation of *A. monosperma* and *A. judaica* as two distinct species and 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 samples of *A. judaica* are diploid with 2n=16 and x=8. Like most other species of *Artemisia* both species have symmetric karyotype but the chromosomes of *A. monosperma* are generally shorter and three populations of this species have a *B. chromosome*, and also that to in Tunisia was addressed by Haouari and Ferchichi,(2008) using ISSR markers; the results indicated patchy distribution to the genetic variability among different populations to this species revealing a contribution of local ecological and geographic conditions on its variability, and Faten *et al.*,(2018) investigation of genetic diversity with RAPD and ISSR markers to *Artemisia herba*, showed that a high level of genetic diversity within populations revealed either with RAPD and ISSR markers (Na^{1/4} 1.67, PPL^{1/4} 66.5%, H^{1/4} 0.26, I^{1/4} 0.38 and Na^{1/4} 1.7, PPL^{1/4} 69.8%, H^{1/4} 0.26, I^{1/4} 0.38, respectively). The level of genetic diversity varied across populations and chemotypes. Populations from the a-thujone/trans-sabinyl acetate chemotype exhibited the highest genetic diversity as revealed by the RAPD markers. However, populations from a-thujone/camphor/b-thujone chemotype showed the important genetic variation determined by ISSR markers.

Table (4):Degree of polymorphism and information content for 5 ISSR primers along with their sequences applied to 3 Artemisia genotype:

N	Primer. N	Sequence (5-3) Forward				% polymorphism (pol/TX100)
			Total (T)	Polymorphi (pol)	Length range (bp)	
1	UBC811	GAGAGAGAGAGA GAGAC	12	7	100-700	50%
2	UBC818	CACACACACACA CAC AG	30	15	100-1000	53%
3	UBC834	GAGAGAGAGAGAGAGAGAT	23	21	100-700	70%
4	UBC849	GAGAGAGAGAGAGAGAT	30	24	100-1000	63%
5	UBC845	CTCTCTCTCTCTG	25	8	100-1000	80%

PIC, Average polymorphic information content for polymorphic bands.

It is worth mentioning that the level to genetic activity could also be due to high mutational rate at ISSR loci which are influenced , used to the structure, length to the repeated units, and also that to the loci with large number to repeated units (ISSR unites) tend to show high mutational rate. Perhaps, most ISSR loci used to my study consisting to hgih number to repeated unites. As a result, any one to the alleles have any mutations may create a heterozygous .

A significant genetic differentiation among populations and among chemotypes was detected.

Especially, Table (4), that genotypes have originated in a relatively small geographical region. So the average to heterozygosity degree across the 5 amplified ISSR loci in this study was fairly low. Moreover, Egyptian Artemisia plants have a narrow genetic base leading of low variability with in the genotypes. These results were comparable to those obtained by Zakkia Gul *et al.*, (2019) where the genetic diversity between *Artemisia* species by estimated using genetic distances (GD) out of genomic, proteomic and morphological documentation and that to Total crude protein concentration in leaves to *Artemisia gemilini* (19.4 %±0.46, range 18.5-20.7 %) and *Artemisia bravifolia* (19.3 %±0.53, range 17.6-20.7 %). SDS-PAGE revealed specific protein bands only in *A. gemilini*. Total genomic DNA extracted from bark using optimized isolation protocol., adipdcution our results are with Mona and Najat,(2014). They studied genetic diversity between populations and a dendrogram constructed to in agreement by inter-simple sequence repeat (UBC810, UBC811,UBC818, UBC834 and UBC849), the results gave the best amplification which produced a total of 43 polymorphic bands and the number of polymorphic loci was 20 and the percentage of polymorphism was 90.47 %, so the similarity result indicates the presence of high level genetic diversity between populations and a dendrogram constructed by UPGMA method, and also to Mohammad *et al.*,(2018) investigation Studies on the genetic variation in marginal populations and differentiation between them are essential for assessment of best gene conservation strategies

and sampling schemes to *Salvadora persica* , by used to ISSR markers ,so that to The levels of genetic diversity maintained within populations of *S. persica* indicate that an appropriate sampling design for ex situ safeguarding should capture the majority of genetic diversity found within these taxa to help ensure the long term viability of this species. Furthermore, it could be inferred that ISSR markers are suitable tools for the evaluation of genetic diversity and relationships with in the *Salvadora persica*.(Table 5).

Table (5):survey of SSRs ramplicons across Five plants of *Artomisia monpsperma* using SSRsr primer.

DNA Marker	Size (bp)	Compostate gene type		
		1	2	3
UBC811	100-700	1	2	3
UBC818	100-1000	1	1	1
UBC834	100-700	1	1	1
UBC849	100-1000	1	1	1
UBC845	100-1000	1	1	1
Total	5	5	5	5

where (1) means presence (0) means absence and shading means specific marker .

3.2.Genetic fingerprints for discrimination and conservation to the Egyptian *Artemisia monosperma* genotypes

The average to matching fingerprints between the three Egyptian *Artemisia monosperma* genotypes of the level of all loci, confirms the intermediate degree to similarity between them. A possible explanation is that these genotypes derived from seeds reproduction. Taking into account,existence of a small proportion to out-cross pollination.Whereas, genetic segregation is more effective in the emergence to species diversity than domestication .

The genetic analysis to the 3 *Artemisia* sp genotypes based on 5 ISSR markers detected 3 distinct specific alleles.

Three genotypes (G.1, G.2 and G.3) had alleles were unique. Wherever, five specific alleles considered as positive specific/unique alleles ⁽⁺⁾, (Table 5). So these five genotypes did not give identical DNA-fingerprints. The non-unique polymorphic band research resulted for the five amplified loci. And these bands that to provide further ability of distinguish between the studied *Artemisia* plant genotypes, through presence or absence to these bands. Consequently, none of the pairs to *Artemisia* genotypes in the present research considered as identical genotypes. On the other side, sign up 12 unique alleles to in the time study can be accompanied with a number to novel functional alleles. This can be used of the improvement to Egyptian *Artemisia*.

So, modern molecular techniques could provide more information for discovering the genetic diversity among Egyptian *Artemisia* as bioenergy crop, aromatic and medicinal plant at the level of DNA. Also, the combination between morphology and molecular description can give trend for conservation and breeding purposes.

3.3. The amplified alleles from ISSR loci and association with genes responsible for oleic acid content , (Table 6).

Table (6): Heterozygosity grade and specific/Unique alleles within genotypes under study

No	Genotypes	Heterozygosity	Specific/Unique alleles ^(+ or -)
1	G1	0.8	UBC811_400bp(-), UBC 818_700 bp(+) UBC834_1000 bp(+), UBC849_1000bp(+) UBC845_2000bp+
2	G2	0.34	
3	G3	0.64	
	Mean	0.34	

⁽⁺⁾ indicates that the unique alleles are positive or present but ⁽⁻⁾ negative unique alleles.

3.4. Genetic similarity and clustering of the genotypes

Among the several co-dominant markers, the ISSRs have several advantages due to their co-dominant nature, are abundant in the genome, are highly reproducible, possess hyperpolymorphism, are rapidly evolving, and can be applied both on an infraspecific level and between related species. Table (7): Similarity indices (%) calculated by Dice computer package among ten *Artemisia monosperma* genotypes using five ISSR primers :

Genotypes	G1	G2	G3
G1	1.00		
G2	0.50	1.00	
G3	0.65	0.67	1.00

As the pedigree data of the analyzed genotypes in the present study are not available. Because, mating system in *Artemisia* is normally self-pollination with a very limited proportion of out cross pollination. While, on the other hand the lowest similarity degree was 0.67 between genotypes number 2 and 3, also was low comparatively between genotype number 2 and most rest of the genotypes (Table 7). This means that these genotypes have the highest genetic distance and thus differ genetically from each other. There may be some reasons to explain this reduction. It is likely that the genetic diversity developed even among clonal offspring of the same cultivar and this is the result of accumulation of somatic mutations over many years. It can't disregard role of the genetic segregation after limited proportion of the out cross-pollination in the main source of these genotypes. All of that would have caused release of several lines genetically different which might have originated from the same source. In the present study, the genotypes under study were clustered and the dendrogram could be divided into four sections or clusters. Cluster 1 consisted of four genotypes (G.1, G.2, and G.3). Genotypes 7 and 10 showed the highest genetic similarity

and formed a separate sub cluster. Also, G.1 and G. 5 were very closely related to each other.

Data based on Average Linkage (Between Groups), using Similarity computed according to Dice coefficient, with 40 fragments. Whereas, Cluster consisted also of three genotypes (genotypes 1, 2 and 3); it was observed that three genotypes were very closely related to each other, especially genotype 1 with 2 (0.63). Where, both genotypes showed high genetic distance with the rest of *Artemisia* genotypes. Especially genotype 3 showed highest genetic

distance with the rest genotypes under study. In fact, the dendrogram generally indicates the genetic relationships among set of genotypes in a population and may express the evolutionary and parentage history for thus genotypes. This is in a case that the samples of population under study were numerous and diverse enough as reported by Tran M.H., *et al*(2004). Consequently, the dendrogram in the present study was just to assessment structure of the genetic variation not to understand any evolutionary relationships. In conclusion, we can conclude out of results this part of the present study. In the three ISSR markers detected mode rate number of the alleles per locus and high percentage of polymorphism. The average of heterozygosity degree in the genotypes and across the 5 amplified ISSR loci in this study was fairly low. It has been pointed out that one of the reasons for this, the nature of amplified ISSR loci that most of them are located in transcribed regions or expressed sequence tags (ISSR) not genomic DNA sequences. Consequently, the performance of agiven ISSR markers to measure the polymorphism. In this context, some alleles or bands were identified in the genotypes under study and matched to those identified in previous studies, for example alleles associated with toxicity.

4. REFERENCES

- Abdelfattah B., Wafaa M., Sahar A., Sahar S. and Amal S.,(2012). Genetic diversity in *Artemisia monosperma* and *Artemisia judaica* populations in Egypt based on morphological, karyological and molecular variations., *Journal of Medicinal Plants Research* Vol. 6(1), pp. 66-78, 9.
- Bodian A., Mohamed A. and Khadidiatou N. (2012). Genetic diversity analysis of date palm (*Phoenix datylifera* L.) cultivars from Figuig oasis (Morocco) using SSR markers. *International Journal of Science and Advanced Technology*. 2 (3):96-104.
- Faten Y., Najoua R., Sameh M., Mohamed B. and Chokri M., (2018). Relationship between chemotypic and genetic diversity of natural populations of *Artemisia herba-alba* Asso growing wild in Tunisia., *Phytochemistry* 148: 48-56.
- Gadaleta A., Mangini G., Mulè G. and Blanco A. (2007). Characterization of dinucleotide and trinucleotide EST-derived microsatellites in the wheat genome., *Euphytica J.*, 153:73-85.
- Haouari M. and Ferchichi A., (2008). Study of genetic polymorphism of *Artemisia herba-alba* from Tunisia using ISSR markers., *African Journal of Biotechnology* Vol. 7 (1), pp. 044-050.
- Khaled E. and Abdelrezak E., (2019). Genetic Diversity of *Artemisia herba-alba* in Libyan Green Mountain., *Iranian Journal of Science and Technology, Transactions A: Science*, 43:1507–1512.
- Mohammad A. M., Davood S., Gholam R. S.S. and Fatemeh S., (2018). Assessment of genetic diversity in *Salvadora persica* L. based on inter simple sequence repeat (ISSR) genetic marker., *Journal of Genetic Engineering and Biotechnology.*, (16): 661–667.
- Mona S. W and Najat A. B., (2014). Inter-simple sequence repeat (ISSR) analysis to molecular characteristic determination of *Heliotropium digynum* in Saudi Arabia., *International Journal of Advanced Research*, Volume 2, Issue (10): 902-906.
- Ohsawa T. and Ide Y (2008). Global patterns of genetic variation in plant species along vertical and horizontal gradients on mountains. *Glob Ecol Biogeogr* 17:152–163.
- Tanya P, Taerprayoon P, Hadkam Y, Srinives P (2011) Genetic diversity among *Jatropha* and *Jatropha*-related species based on ISSR markers. *Plant Mol Biol Rep* 29(1):252–264
- Tran M.H., Nguyen T. P. T., Nguyen T. T., Tran H.T., (2004). *Artemisia vulgaris* L. from Vietnam: Chemical Variability and Composition of the Oil Along the Vegetative Life of the Plant., *Journal of Essential Oil Research* V16(4):358-361.
- Vischi M., Cristina., Steluta R., Luca P. and Rachele M., (2017). Genetic Diversity of Walnut (*Juglans Regia* L.) the in Eastern Italian Alps., *Forest Articial.*, V8, P81.
- Paolo Ermacora 1, Guido Cipriani 1, Donatella Paffetti 3,4, Cristina Vettori 4 and Raffaele Testolin 1 Viruel M. A., Escribano P., Barbieri M., Ferri M. and Hormaza J. I. (2005). Fingerprinting, embryo type and geographic differentiation in mango (*Mangifera indica* L., Anacardiaceae) with microsatellites. *Molecular breeding*. 14: 383-383.

Viruel M.A., Escribano P., Barbieri M., Ferri M. and Hormaza, J.I. (2005). Fingerprinting, embryo type and geographic differentiation in mango (*Mangifera indica* L., Anacardiaceae) with microsatellites. *Mol. Breed.* 15: 383–393.

Zakkia G., Imtiaz A. K., Chandni K. and Tika K., (2019). Comparative genomic, proteomic and morphological studies in two indigenous artemisia species collected from high mountains of Karakorum and himalaya in Gilgit Baltistan., *Plants & Agriculture Research*, V(9), Issue 1.

تحديد الإختلافات الجينية بين نباتات الأرماسيا مونوسبيرما *Artemisia monosperma* باستخدام البصمة الوراثية

أحمد نشأت محمد العظلة – وردة يوسف على

شعبة البيئة وزراعات المناطق الجافة - مركز بحوث الصحراء - المطرية - القاهرة - مصر

ملخص

في هذه الدراسة ، تم اختيار ثلاثة طرز وراثية مصرية من نبات *Artemisia monosperma* من مدينة بئر العبد بشمال سيناء ، مصر، للتحليل الوراثي باستخدام 5 من معلمات تكرار التسلسل البسيط (ISSR). وأظهرت النتائج المتحصل عليها أن بادئات ISSR قادرة على مضاعفة المواقع الوراثية تحت الدراسة ، وكشفت تعدد أنماط الحمض النووي بين الطرز الجينية. تشير البيانات إلى أنه من إجمالي 120 نمط ، كان 64 نمطا متعدد الأشكال (64%). وتراوحت عدد النطاقات المرصودة من اثنين للباديء UBC849 إلى واحد UBC811 بمتوسط 2.4 عبر التراكيب الجينية المختبرة. كما أظهرت النتائج وجود عدد معقول للأليلات لكل موقع في الدراسة الحالية ، قد يرجع سبب ذلك الى طبيعة مواقع ISSR المكبرة التي يقع معظمها في علامات التسلسل المعبر عنها في تسلسل الحمض النووي الجيني للمناطق المنسوخة وبالتالي فإن معظم مواقع ISSR الخمسة في هذه الدراسة كانت مفيدة لتقييم التنوع الجيني بين التراكيب الجينية المصرية الثلاثة من نبات *Artemisia monosperma*. ومع ذلك ، تجدر الإشارة إلى أن هذه القيمة تعبر عن وجود أليلات مختلفة لموقع واحد أو أكثر من الكروموسومات المتجانسة. تشير قيمة محتوى بيانات تعدد الأشكال (PIC) لـ ISSR الجينومي إلى مستوى عالٍ إلى فاعلية الشكل بمتوسط قيمة PIC إلى 0.42 على جميع بادئات ISSR المختبرة. حيث تراوحت من 0.07 باستخدام UBC849 إلى 0.42 في UBC811 لجميع التركيب الوراثي قيد البحث بمتوسط 0.18. كما توصلت الدراسة إلى مستوى منخفض من تغاير الأنماط الجينية تحت الدراسة ، بحيث يكون الاختلاف بين عدد الأليلات لكل موضع و عدد الأليلات الفعالة ، بحيث تكون موجودة لأليلات حصرية / محددة للأنماط الجينية. كشف التحليل الجيني لثلاث أنواع من *Artemisia monosperma* عن 64 أليلات محددة منفصلة. وكذلك تشير النتائج إلى وجود أليلات ثلاثة (G.1 و G.2 و G.3) كانت منقطعة النظير. لذا فإن هذه الأنماط الجينية الخمسة لا تعطي بصمات دنا مطابقة. وتجدر الإشارة إلى أن أكبر عدد الأليلات المحددة / الفريدة كان من التركيب الجيني رقم 3.

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