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Assessment of the Genetic Diversity of Some Female Jojoba (*Simmondsia chinensis* (Link) Schneider) Genotypes Growing in the Semi-arid Highlands of Sudan using ISSR Markers

*Ismail A. Mohammed[1], Rania S. Elsanousi[1], Lana A. Ibrahim[1], Ramah A. AbdAlgadir[1], Sayeda O. Elhoweiris[1], Fatima M. Osman[1],*Seif M. Gasim[2]

[1]Department of Botany and Agricultural Biotechnology, Faculty of Agriculture, University of Khartoum, Khartoum North, Shambat, P.O. Box 32, Postal Code 13314, Sudan.
[2]Department of Agronomy, Faculty of Agriculture, University of Khartoum, Khartoum North, Shambat, P.O. Box 32, Postal Code 13314, Sudan.
***corresponding authors:ismaildamer@gmail.com;seifm1963@gmail.com**
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Abstract: The knowledge of genetic diversity of a crop like Jojoba, a commercially grown plant to produce unique liquid oil from its seed, is essential in a breeding program aiming to the development of elite genotypes for large-scale production. This study was carried out to evaluate the genetic diversity of 25 female jojoba genotypes growing in Arkaweet Area, East Sudan, using ISSR Markers. Genomic DNA was extracted from leaves of the studied genotypes and quantified for PCR amplification. Five ISSR primers were used for PCR amplification to investigate the genetic diversity among the female jojoba genotypes. ISSR primers showed high polymorphic pattern and produced a total of 54 polymorphic bands with an average of 10.8 polymorphic bands per primer. The average of polymorphic information content (PIC) value of 0.37 indicates moderate polymorphism. The genetic diversity ranged from 0.01 to 0.85. The results indicate the effectiveness of using ISSR molecular markers to reveal genetic variability among the studied female jojoba genotypes, a finding that could be used for development of elite jojoba genotypes, which provide potential for large-scale jojoba production in Sudan.

Keywords: jojoba;genetic diversity**;** liquid wax;ISSR marker; polymorphism; Sudan.

INTRODUCTION

Jojoba, *Simmondsia chinensis* (Link) Schneider is dioecious, perennial,ever-green desertshrub belonging to Simmondsiaceae. It is native to the Sonoran Desert of Arizona and arid California of the United States, as well as Northern Mexico (Modise 2007; Osman and Abohassan 2013). Jojoba is a non-edible oil seed plant growing in extreme environments of annual precipitation of 380-450 mm, high salinity and temperatures of 43 ° - 46 ° C as reported byHogan (1979), which makes jojoba a key crop in the context of global climate change. The plant is widely cultivated in many countries in South America, Africa, India, the Middle East and Australia for its seed oil (Reddy and Chikara 2010; Aly and Basarir 2012). Jojoba seed contains unique oil of the plants of the world that make up to 65% of seed weight (Miklaszewska and Banaś 2016). Its oil is a liquid wax which is totally different from other vegetable oils. The oil, being a poly-unsaturated liquid waxfree from glycoside esters, is similar to that of sperm whale. Such oil is used in several industrial applications such as lubricants, cosmetics, pharmaceuticals,waxes, detergenets and biodiesel (Wisniak 1994),thus contributes to restricting whale hunting (Hill and Hofer 2009).Female plants produce the seeds containing the oil, thus selection of elite female genotypes is crucial for the production of the valuable liquid wax.

Sudan is considered as a pioneer country among Arabian and African countries in introducing jojoba. The crop was introduced in the late seventies by UNDP in a joint venture program with Sudanese government. The main objective of the program was to introduce a new cash crop that suits the less advantageous areas (Osman 1983). Accordingly, seven sites were selected on the basis of their close similarities to the natural habitat of the plant and representing various climate and soil conditions in the Sudan. Although Sudan is not within the belt where jojoba is grown naturally, the plant has been established successfully and developed seeds, particularly in Arkaweet

(Osman 1983). The plantation established in late seventies is still thriving,drawing attention to researchers and investors that the country could have a real potential investment possibility for production of seeds and oil. Therefore, understanding the genetic diversity in the available germplasmrepresents the basis for improvement of the crop species. For the assessment of genetic diversity, molecular markers (PCR-based and hybridization- based markers) have been used(Arya *et al*. 2016; Inoti 2017; Agarwal and Khan 2018). Among the molecular markers, Inter Simple Sequence Repeats (ISSR), PCR-based markers have been used by many workers (Heikrujam *et al.* 2014; Agarwal and Khan 2018). This marker is fast, simple, not affected by environment and do not require any prior knowledge about the sequences to be amplified. There is a lack of information about the evolutionary and genetic relationships among different jojoba genotypes growing in Sudan using molecular marker techniques. In addition no research work has been undertaken to investigate the genetic diversity of jojoba plants in Sudan, despite its early introduction.The present study was conducted to evaluate genetic diversity among some jojoba female genotypes growing in Arkaweet using ISSR markers.

MATERIALS AND METHODS

The study was conducted at the molecular biology laboratory, Department of Botany and Agriculture Biotechnology, Faculty of Agriculture University of Khartoum in 2017-2018.

Plant materials:

Young leaves from twenty five female jojoba genotypes were collected from Arkaweet,Red Sea state, Sudan and used for DNA extraction (Table 1).

Genomic DNA extraction:

Genomic DNA was isolated from young leaves of female jojoba genotypes using modified CTAB method of Saghai-Maroof *et al.* (1984). Leaf samples were ground to fine powder using liquid nitrogen in sterilized mortar and pestle. Five grams of tissue powder were mixed with 10 ml of pre-warmed

CTAB extraction buffer at 65°C plus 2 % PVP (Polyvinylpyrrolidone) in sterilized 15 ml Falcon tubes and shacked several times. The mixture was incubated in water bath at 65°C for one hour. Contents of the tubes were gently mixed at an interval of 10 minutes by inverting them several times. After incubation, the samples were cooled for 5 minutes at room temperature and then 10 ml chloroform: iosamylalcohol (24:1) mixture was added. The mixture was mixed gently by inverting the tubes several times. The samples were centrifuged for 10 minutes at 8000 rpm in (Hettich MIKRO 220R, Germany) at room temperature. The upper aqueous layer was transferred to a new sterilized Eppendorf tube. The extracted DNA was precipitated with equal volume of ice cold isopropanol followed by centrifugation at 10000 rpm for 5 minutes and then the upper aqueous layer was dropped out. DNA pellets were washed twice with Wash I solution (Ethyl alcohol 76.0 % and 0.2 M Sodium acetate (pH 5.2)) for 20 minutes followed by Wash II solution (10 mMTris (pH 8.0) and 1 mM EDTA (pH 8.0)) for 2 minutes, and centrifuged for 5 minutes at 4000 rpm in each. DNA samples were dried at room temperature for one hour and subsequently dissolved in appropriate volume of sterilized double distilled water.

RNase A treatment:

DNA samples were treated with RNase Asolution (50 µg/ml) and incubated at room temperature for overnight to remove RNA contamination from DNA samples. DNA was again extracted by adding an equal volume of chloroform: isoamylalcohol (24:1 v/v) mixture. Samples were mixed well and then centrifuged at 10000 rpm for 10 min. Supernatant was transferred to new Eppendorf tubes. 1/10 vol. of 3M sodium acetate (pH 6.8) and 2 vol. of chilled absolute alcohol were added, followed by centrifugation to pellet down the DNA. Pellet was then washed with 70% alcohol, air-dried and finally dissolved in 50 µl of sterilized double distilled water. DNA concentration and purity were measured using Nanodrop(ND-1000 UV-Vis Spectrophotometer, USA).

PCR amplification:

DNA samples were diluted to 5ng/µl before used for PCR amplification. Five ISSR primers (UBC811, UBC818, UBC807, UBC827 and UBC830) were used for PCR amplification of genomic DNA from the twenty five female jojoba genotypes (Table 2). PCR reactions were carried out in 20µl volume containing 2µl of DNA template, 0.4µl of each primer (10-20 picomole) and 4µl of 5x FIREPol Master Mix (Solis BioDyne). PCR conditions for UBC811 and UBC818 primers were94°C for 5 minutes as an initial step, followed by 40 cycles of 1 minute at 94°C for denaturation, 1 minute at 60°C for annealing and 1.5 minutes at 72°C for extension, and a final cycle of 7minutes at 72°C.While the PCR conditions for UBC 807, UBC827 and UBC830 primers were 94°C for 3 minutes as an initial step, followed by 35 cycles of 20 second at 94°C for denaturation, 1 minute at 50°C for annealing and 1.5 minutes at 72°C for extension, and a final cycle of 7minutes at 72°C. PCR products were then separated by gel electrophoresis on 0.8 % agarose gels, stained with ethidium bromide (5 µl) in a 1x TBE and 8µl of each sample was loaded in each well. Electrophoresis was conducted for 45 minutes at 100 voltages at 400 mA. DNA fragments were visualized with the UV trans-illuminator in the presence of 100 bp DNA ladder.

Data analysis:

PCR products were scored visually and recorded as (1) for the present band and (0) for the absent band. Polymorphic information content (PIC) value was calculated according to Roldan-Ruiz *et al*. (2000) as:

$PIC = 2fi(1 - fi)$

Where, fi is the frequency of the amplified allele (band present), and (1− fi) is the frequency of the null allele (band absent) of amarker.

A dendrogram was constructed using UPGMA (Unweighted Pair Group Method with Arithmetic mean) with hierarchical clustering method in PAST3 software (Hammer *et al*. 2001) to determine genetic relationships among jojoba genotypes by similarity.

RESULTS AND DISCUSSION

Due to the absence of genetic information on the genetic diversity of the present grown female jojoba genotypes at Arkaweet, the use of molecular markers for assessing the genetic diversity of such genetic material is worthwhile as Jojoba plant has been successfully established and developed seeds under extreme environmental conditions. In the present study, the concentration of extracted DNA from jojoba genotypes ranged from 8.95 to 3427.75 ng/µl, while the purity ranged from 1.00 to 2.78 (Table 1). The results provided a good quality and quantity of extracted DNA for PCR amplification. Table 1 shows the concentration and purity of extracted DNA from twentyfive female jojoba genotypes used in this study. The results in the table revealed good quality of DNA for PCR amplification. Aly and Al-Badawy (2005) reported that a few primers could successfully produce amplification pattern and polymorphism among jojoba genotypes.

Table 1**.** Concentration and purity of extracted DNA from twenty five female jojoba genotypes.

S. No	Jojoba Genotype	DNA conc (ng/µl)	DNA purity	S. No	Jojoba genotype	DNA conc (ng/µl)	DNA purity	S. No	Jojoba genotype	DNA conc (ng/µl)	DNA purity
1	JoSARF1	59.57	1.96	10	JoSARF14	40.26	1.6	19	JoSARF30	70.94	1.55
2	JoSARF2	8.95	1.47	11	JoSARF15	12.7	1.83	20	JoSARF31	43.55	1.85
3	JoSARF3	199.32	1.86	12	JoSARF16	151.71	1.83	21	JoSARF32	44.14	2.78
4	JoSARF5	32882.	2.5	13	JoSARF17	1341.2	1.9	22	JoSARF33	20.36	2.22
5	JoSARF6	477.61	1.73	14	JoSARF20	12.66	1.6	23	JoSARF35	71.32	1.73
6	JoSARF7	216.67	1.51	15	JoSARF24	92.91	1.83	24	JoSARF36	112.64	1.62
7	JoSARF8	88.42	1.71	16	JoSARF25	72.03	1.85	25	JoSARF38	111.43	1.55
8	JoSARF11	278.43	1.58	17	JoSARF27	2299.12	1.35				
9	JoSARF12	244.67	1.00	18	JoSARF29	3427.75	1.83				

In this study, all primers showed polymorphic pattern and produced a total of 54 polymorphic bands among the jojoba genotypes (Table 2; Fig. 1). The molecular weight of amplified fragments was in the range from 190 to 1400 bp. Primer UBC807 produced the highest (16) number of polymorphic bands, while primer UBC827 produced the lowest (7) number of polymorphic bands. The average of polymorphic bands per primer was 10.8 (Table 2; Fig. 1).These imply that the studied female jojoba genotypes are highly polymorphic and ISSR maker can be considered as an effective molecular tool to identify polymorphism among female jojoba genotypes. The dinucleotide repeat (GA)n and (AG)n primers amplified the highest number of bands (Table 2), which reflects its redundancy in jojoba genome as reported by Al-Soqeer *et al*. (2012).The results of ISSR data showed a PIC value in the range from 0.30 to 0.44with an average of 0.37(Table 2), which indicate the presence of polymorphism among the studied female jojoba genotypes. The present PIC value is higher than the range of PIC value obtained by Bhardwaj *et al.* (2010) in other study of jojoba female genotypes using RAPD and ISSR markers. The genetic diversity was estimated by cluster analysis by grouping similar genotypes in one group. The genetic similarity among the studied genotypes ranged from 0.15 (JoSARF8, JoSARF5 and JoSARF38) to 0.99 (JoSARF11 and JoSARF12).The dendrogram (Fig 2.) delineated three main clusters; the first cluster contains 20 genotypes and was divided into two sub-clusters. The first sub-cluster divided into two groups. The first group comprised the genotypes JoSARF (14 and 20) with a genetic similarity of 0.61. The second group comprised the genotypes JoSARF (25, 30 and 31) with a genetic similarity of 0.69.

Table 2. ISSR primers sequences and amplification efficiency of twenty five Female jojoba genotypes.

S. No	ISSR primer	Sequences (5′ → 3′)	Range of amplified fragments (bp)	Total number of bands	Poly morp hism %	PIC
1	UBC807	AGA GAG AGA GAG AGA GT	1400 – 190	16	100	0.44
2	UBC811	GAGAGAGAGAGAG AGAGAC	1400 – 300	13	100	0.30
3	UBC818	CACACACACACAC ACAG	1100 – 290	8	100	0.32
4	UBC827	CACACACACACAC AGAG	1300 – 390	7	100	0.43
5	UBC830	GTGTGTGTGTGTGG	1400 – 300	10	100	0.37
Avera ge				10.8		0.37

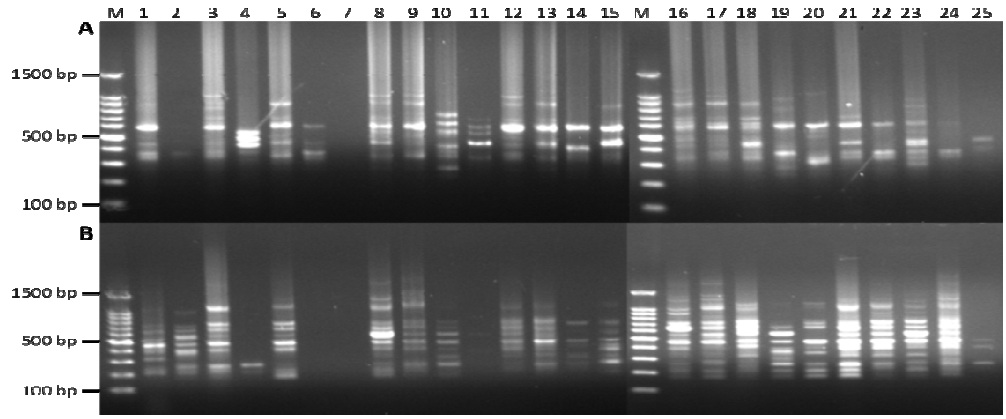

Fig. 1. PCR amplification of 25 female jojoba genotypes using UBC811 (A) and UBC807 (B) primers, M: marker, Lanes 1 – 25 representJoSARF1 - JoSARF25 genotypes.

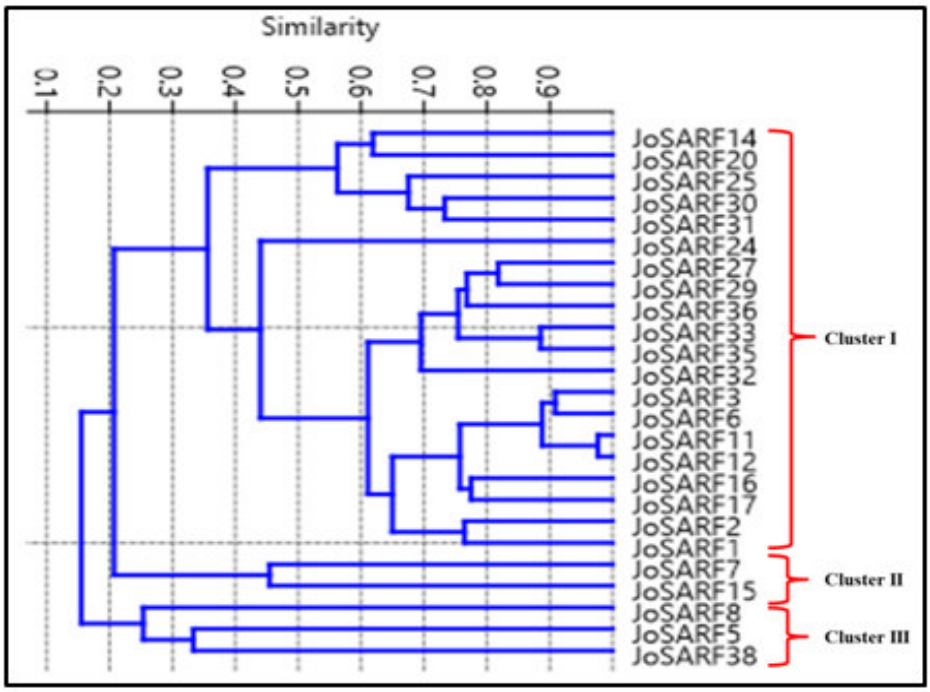

Fig. 2. Phylogenetic tree of 25 female jojoba genotypes

The second sub-cluster consists of three groups. Group I comprised the genotype JoSARF24 with a genetic similarity of 0.45. Group II divided into three subgroups. Subgroup I comprised the genotypesJoSARF(27, 29 and 36)with a genetic similarity of 0.77. Subgroup II comprised the genotypes JoSARF (33 and 35) with a genetic similarity of 0.89. Subgroup III comprised the genotypes JoSARF(32) with a genetic similarity of 0.70. Group III divided into four subgroups. Subgroup I comprised the genotypes JoSARF (3 and 6) with a genetic similarity of 0.91. Subgroup II comprised the genotypes JoSARF (11 and 12) with a genetic similarity of 0.99. Subgroup III comprised the genotypes JoSARF (16 and 17) with a genetic similarity of 0.78. Subgroup IV comprised the genotypes JoSARF (2 and 1)with a genetic similarity 0.77.The second cluster comprises the genotypes

JoSARF (7 and 15) with a genetic similarity 0.46. The third cluster was divided into two groups. The first group comprised the genotype JoSARF8 with a genetic similarity of 0.25, while the second group comprised the genotypes JoSARF (5 and 38) with a genetic similarity of 0.33.The range of similarity of 0.15 to 0.99 as well as the classification of female jojoba genotypes in different groups indicate the presence of high genetic diversity among the genotypes under the study. Similar results were reported by different authors (Gaber *et al.* 2007;Sharma *et al*. 2009; Al-Soqeer 2010; Heikrujam *et al*. 2015).Such level of high genetic diversity could be used in the development of elite jojoba genotypes for large-scale production in Sudan, especially after solving the problem of sex determination at the early stage of seedling (Heikrujam *et al.* 2014;Mohamed *et al*. 2019). This high level of genetic diversity may be due to introduction of different female jojoba genotypes from different countries to the study area. However, Osman and AboHassan (2013) reported moderate genetic diversity among jojoba clones selected in Makkah of Saudi Arabia, whereas Inoti (2017) found low genetic diversity among female jojoba genotypes of semi-arid areas of Kenya using SSR markers. The results of the present study represent the first report on the evaluation of the genetic diversity of female jojoba genotypes in Sudan using ISSR marker.

CONCLUSION

- Based on the obtained results, it is concluded that the DNA molecular marker (ISSR) is effective.
- The information about the genetic diversity of the genotypes can facilitate the selection of parents in breeding programs to develop elite genotypes to be used for large-scale jojoba production in Sudan.
- Such information together with the study of drought and salinity tolerance along with the valuable oil of the species, jojoba may replace some of our conventional agricultural crops, especially non-edible oil plants.

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تقييم التنوع الوراثي لبعض الطرزالوراثية المؤنثة للجوجوبا النامية فى الأراضي المرتفعة شبه الجافة بالسودان بإستخدام واسمات ISSR

اسماعيل أحمد محمد[1*]، رانيا سليمان السنوسى[1]، لنا على ابراهيم[1]، رماح عبدالرحمن عبدالقادر[1]، سيده عمرالحويرص[1]، فاطمة مكاوى عثمان[1]، سيف مضوى قاسم[2]

[1] قسم النبات والتقانة الحيوية، كليةالزراعة، جامعة الخرطوم، السودان
[2] قسم المحاصيل الحقلية، كلية الزراعة، جامعة الخرطوم، السودان

المستخلص: يعد تقييم التنوع الوراثي لمحصول مثل الجوجوبا ، نبات يزرع تجاريًا لإنتاج زيت سائل فريد يستخلص من بذوره، أمرًا ضروريًا في برنامج التربية الذي يهدف إلى تطوير أنماط وراثية متميزة للإنتاج على نطاق واسع. أجريت هذه الدراسة لتقويم التنوع الوراثي لخمسة و عشرين طرازاً وراثياً مؤنثاً من نبات الجوجوبا الذى ينمو في منطقة أركويت بشرق السودان باستخدام واسمات جزيئية داخلية بسيطة متكررة (ISSR) . إستخلص الحمض النووى الجينومى من أوراق الطرز الوراثية قيد الدراسةوحددت كميته لتفاعل البلمرة المتسلسل (PCR).أستخدمت خمس بادئات من ISSR فى تفاعل البلمرة المتسلسل (PCR) للتحقق من التنوع الوراثي مابين الطرز الوراثية المؤنثة لنبات الجوجوبا. أظهرت بادئات ISSR قيم عالية لمحتوى المعلومات متعددة الاشكال (PIC) و أنتجت 54 حزمة متباينة بمتوسط 10.8 حزمة لكل بادئ. متوسط قيم محتوى المعلومات متعددة الاشكال0.37 مشيراً الى تباين وراثي معتدل. وتراوح قيم التنوع الوراثي من 0.01 إلى 0.85. تشير النتائج الى فعالية واسمات ISSR فى كشفالتباين الوراثي بينالطرز الوراثية المؤنثة لنبات الجوجوبا قيد الدراسة . هذه النتائج يمكن استخدامها لانتاج نخبة من الطرز الوراثية لنبات الجوجوبا للانتاج على نطاق واسع فى السودان.