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Molecular Analyses of Genetic Diversity in the Population of *Calligonum comosum* L. 'Her in Hail Region in the Kingdom of Saudi Arabia

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Abstract: ***This paper presents the first molecular study on Calligonum comosum L. 'Her (Polygonaceae) in the Kingdom of Saudi Arabia. It's known to be part of Saudi Arabia's flora and is a famous medicinal plant. And it has been included on the endangered species list due to its frequent use. Twenty - five C. comosum samples were collected from five different populations in their natural environment and screened using Inter - Simple Sequence Repeat (ISSR) markers. Eleven ISSR primers amplified 975 reproducible bands, of which 495 were polymorphic, and the polymorphism of genetic variation between species was 50.8%. This ratio is considered high Among C. comosum members from multiple populations, this ratio is an indication of the quality of genetic diversity within and between C. comosum populations, which is of great importance for the preservation of the plant. As considered, ISSR markers are an effective tool to provide good information about the genetic diversity available in C. comosum inputs from different populations, and the results of this study will help to understand genetic variation and evolutionary dynamics in C. comosum and expand the genetic base for breeding wild species. That additional selection of inputs in each species to assess genetic diversity leads to more useful and reliable results.***

Keywords: *Calligonum comosum*, ISSR, Polymorphism, noiger liaH**,** Saudi Arabia

1. Introduction

Calligonum comosum L`Her (Polygonaceae) is a wild plant known as part of Saudi Arabia's flora (Chaudhary and Al - Jowaid, 1999). It plays an essential role in stabilizing sand due to the large root system that characterizes the plant; it is very branching and fast - growing, as its roots reach great depths in the soil, reaching thirty meters (Al - Otaibi, 2015). It is a highly palatable species used in forage, medicine, fuel, and dune stabilization to increase soil organic matter content (Ren, 2001; Tao, 2000). *C. comosum* has been included on the endangered species list due to its frequent use. Its species has recently declined as a natural resource due to the public's interest in obtaining it (Liu et al., 2001). Also because of the threats faced by plants in the Kingdom of Saudi Arabia in general, which led to increased fragmentation of habitats such as climate change, deforestation, invasion of some exotic plants, physical and chemical changes in the soil, pollution with various heavy metals, and other abiotic stresses (Khan et al., 2012). Despite the importance of *C. comosum* economically and environmentally, there are no studies available on the genetic diversity of its different populations in some parts of the Kingdom of Saudi Arabia, especially since its population is isolated from each other and the size of some of them is limited, and determining genetic diversity within and between plant populations is of great importance for the improvement of plants. Therefore, knowledge of the genetic diversity of genetic resources and genetic relationships among plants is considered basic and essential information for plant breeders because of its usefulness in designing breeding and improvement programs and in improving the efficiency of genetic asset management and preservation processes from genetic drift (Lombard et al., 2001). This study will provide an estimate of the genetic diversity of inputs from the plant representing their area in the north of the Hail region (Al Nafud Al Kabeer) in the Kingdom of Saudi Arabia, using the inter simple sequence repeats (ISSR) technique, to come up with recommendations to the responsible authorities on how to manage and conserve these populations from environmental degradation or extinction, in preparation for the sustainable use and preservation of their genetic origins. Material and methods

1.1 Plant samples collection

Five *C. comosum* samples were collected from five different populations from their natural environments, from their natural sites in Nafud Al Kabeer region of Hail, Saudi Arabia Table (1) and Figure (1).

1.2 DNA isolation using CTAB method

According to Dellaporta et al. (1983) method, 200 mg of *C. comosum* leaves were ground with liquid nitrogen in a pestle mortar, then the powder was transferred to Eppendorf (2 ml), a solution of 600 µl of extraction solution and 6 µl of RNase was added and mixed for ten minutes. An equal volume of chloroform and isoamyl alcohol 24: 1 is then added, the mixture is transferred to centrifuge at 10000 rpm for 10 min at room temperature, and 300 µl of the supernatant is transferred to the top of the tube to another Eppendorf (1.5 mL).200 µl of ice - cold isopropanol was added, then kept at - 80 ºC for 60 min, after that the mixture was centrifuged at 10000 rpm for 10 min at 4ºC; then the supernatant was discarded. DNA concentration and purityDNA.

Table 1: *C. comosum* specimen collection sites

Sample No.		Location
C1	a	27°52'43.0"N 41°29'26.3"E
	b	27°52'44.5"N 41°29'27.4"E
	c	27°52'44.5"N41°29'27.4"E
	d	27°52'44.8"N41°29'26.9"E
	e	27°52'44.8"N41°29'26.9"E
C2	a	27°52'39.5"N 41°29'24.0"E
	b	27°52'40.5"N 41°29'23.9"E
	c	27°52'40.5"N 41°29'23.9"E
	d	27°52'40.5"N 41°29'23.9"E
	e	27°52'41.8"N41°29'24.9"E
C3	a	27°52'33.9"N41°29'24.8"E
	b	27°52'33.9"N41°29'24.8"E
	c	27°52'33.4"N41°29'25.4"E
	d	27°52'38.7"N41°29'24.5"E
	e	27°52'38.7"N41°29'24.5"E
C4	a	27°52'32.4"N41°29'22.2"E
	b	27°52'32.4"N41°29'22.2"E
	c	27°52'32.2"N41°29'21.5"E
	d	27°52'32.2"N41°29'21.5"E
	e	27°52'32.6"N41°29'23.9"E
C5	a	27°52'30.8"N41°29'20.3"E
	b	27°52'30.8"N41°29'20.3"E
	c	27°52'29.4"N41°29'19.4"E
	d	27°52'28.5"N41°29'18.3"E
	e	27°52'28.5"N41°29'18.3"E

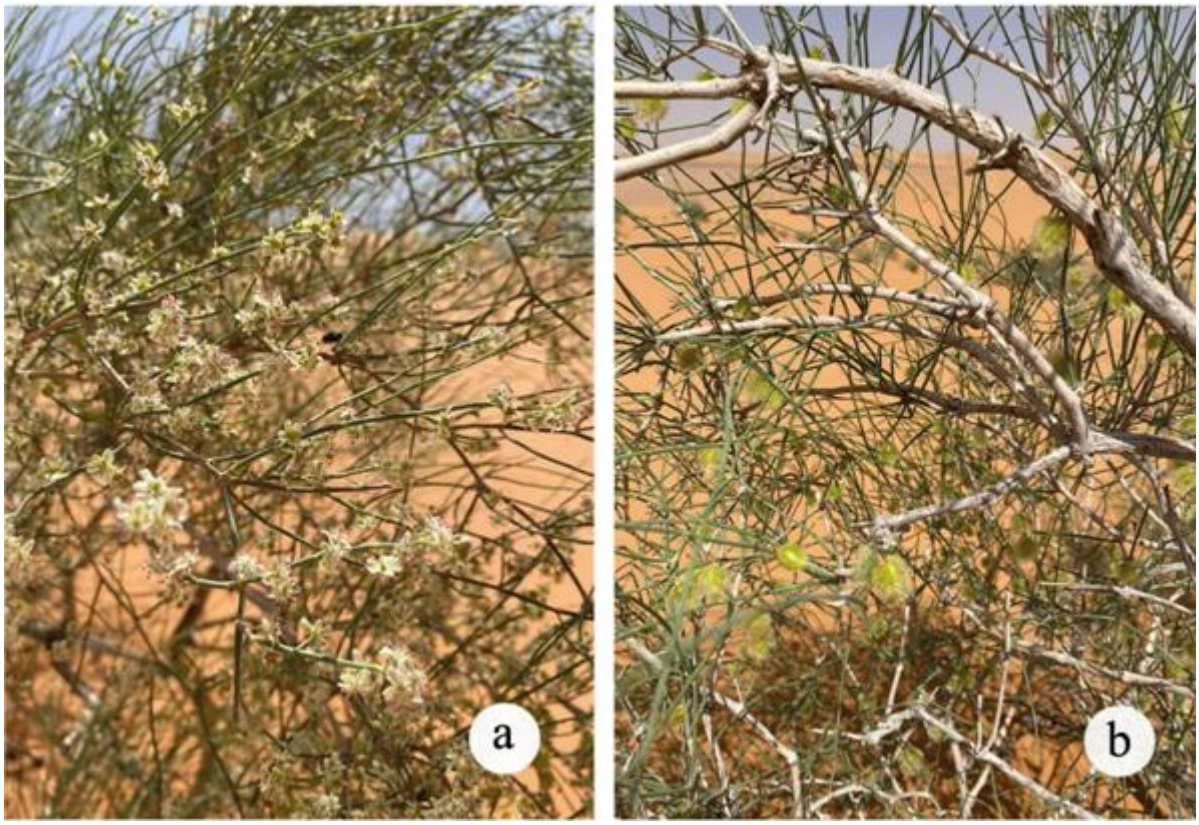

Figure 1: Photographs of *C. comosum*. (a) Flowering stage, (b) fruiting stage (c) whole plant

1.3 Concentration and purity

The quantity and purity of genomic DNA was estimated by NanoDrop spectrophotometer; in beginning, the instrument was set 0 by taking 1µl distilled water as blank. Then, 1.0 µl of the nucleic acid sample was measured at a wavelength of 260 nm, and 280 nm and OD260/ OD280 ratios were recorded to assess the DNA purity, A ratio of 1.8 to 2.0 for OD260/OD280 indicated the good quality of DNA. The purity and quality of the DNA were also checked using 1% agarose gel (1x TBE buffer) stained with ethidium bromide using horizontal gel electrophoresis.

1.4 Preparation of genomic DNA for PCR analysis

DNA samples were diluted to a working concentration of (50 - 60 ng/µl) for PCR amplification.

1.5 PCR reaction for ISSR profiling

The DNA was diluted in doubled distilled water according to concentration, group of 11 ISSR random primers were used (Designed in the lab) sequences and the names of the primers are illustrated in Table (2), The PCR reaction was done in 25 □L volume The doubled distilled water was added in the master mixture followed by ISSR primer and template DNA. The PCR reaction was set in Veriti 96 – well Thermal Cycler. First denaturation at 94∘ C for 3 min, followed by 40 cycles at 94°C for 1min, 48°C for 1min, 72°C for 1min, and final extension at 72°C for 5min, was carried out for the amplification. Agarose gel (1.2%) was prepared in 1x TBE buffer solution for electrophoresis.

Table 2: Sequences of primers that gave clear bundles of DNA with the entries studied of *C. comosum*

remirPon.	DI remirP	ecneuqeS remirP
1.	F USKA1- RSSI	CAACAACAACAACAACAACAA
2.	F-USKA2- RSSI	CACACACACACACACACACAC
3.	F- USKA3- RSSI	ACAACAACAACAACAACAACAA
4.	F- USKA4- RSSI	TCAACAACAACAACAACAACAA
5.	F- USKA5- RSSI	GTTGTTGTTGTTGTTGTTGTTG
6.	F- USKA6- RSSI	CTTGTTGTTGTTGTTGTTGTTG
7.	F- USKA7- RSSI	ATTGTTGTTGTTGTTGTTGTTG
8.	F- USKA8- RSSI	TTTGTTGTTGTTGTTGTTGTTG
9.	F- USKA9- RSSI	GACACACACACACACACACAC
10.	F- USKA10- RSSI	GGTGTGTGTGTGTGTGTGTGT
11.	F- USKA11- RSSI	TACACACACACACACACACAC

1.6 Statistical analysis

The results were statistically analyzed by recording the present or absent band (1, 0) of each sample. The class genetic relationship, similarities, and percentage of polymorphism were calculated and Dendrogram was found among plant samples using Unweighted Pair Group Mean Arithmetic average (UPGMA) to generate a matrix via the using Statistical program Multivariate Statistical Package (MVSP 3.22). This matrix was used to calculate similarity/genetic distance jaccard's coefficient. And the (Freeland et al.2011)

2. Results Dissertation

Eleven ISSR primers were screened to study the genetic relationships among *C. comosum* populations; all the primers produced reproducible polymorphic bands in all five *C.*

comosum populations. In (Figure2 and 2), an image of the ISSR amplification was generated by all primers. The KSU - FAISSR - 2 and KSU - FAISSR - 7 primers produced 150 amplified polymorphic bands was generated across *C. comosum* populations. The number of polymorphic bands was 100 for KSU - FAISSR - 1, KSU - FAISSR - 3, KSU - FAISSR - 7, KSU - FAISSR - 10, and 75 for KSU - FAISSR - 5, and 50 polymorphic bands by the fourth residual primers KSU - FAISSR - 4, KSU - FAISSR - 8, KSU - FAISSR - 9, and KSU - FAISSR - 11. We obtained 975 ISSR bands. The genetic diversity parameters are described in detail (Table 3), 480 of them have identical genetic bundles, while 495 appear to be different. In addition, the percentage of polymorphism was 50.8%.

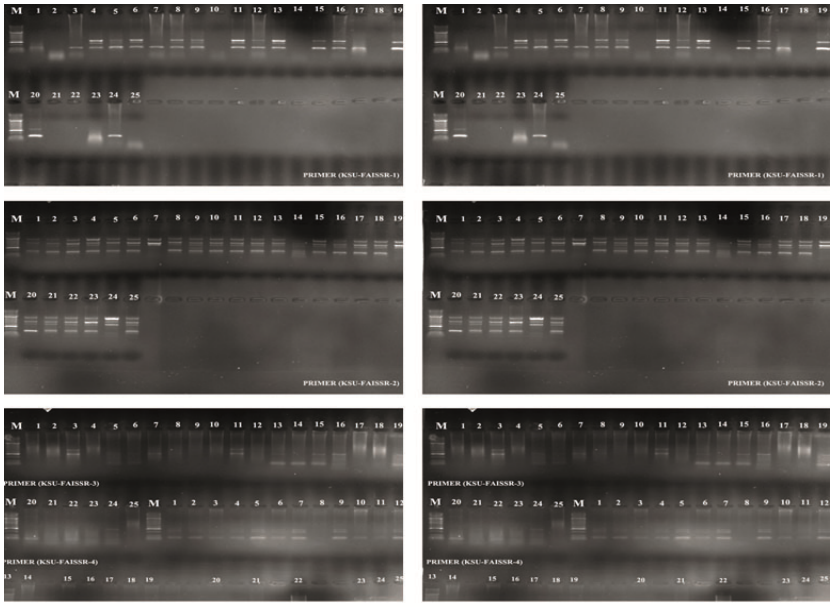

Figure 2: ISSR PCR fingerprints of 25 samples of *C. comosum*

2.1 Genetic relationship between the Hedera helix populations

The number of series, clusters, and groups that carry the studied genetic inputs, and their arrangement in the Dendrogram, as well as the percentage of similarity between them. In (Figure3) showed the Dendrogram obtained during this study using the ISSR technique showed that the cluster analysis was divided into Tow series (2S):

- SI series included all samples from all populations. The second SII sequence included sample C5 - e in a single sequence.
- The first SI sequence was divided into 3 clusters, two of which contained samples from populations C1, C2, and C3, except that the sample C3 - e joined the second cluster, which included samples from populations C4 and C5. The third cluster contains a single sample, C3 - d.

The first and second populations were divided into subclusters gradients in genetic distance.

- It is noticed in the genetic tree that the samples collected from a distance from the center of the population and bearing the symbol e are in a single cluster.

2.2 Similarity matrix

As it is clear from the matrix of the percentage of similarity for the results of ISSR Table (4), that the sample C5 - e has given a percentage of similarity with other samples equal to zero, so this sample came in a single sequence in the kinship tree, but in terms of the genetic dimension between the remaining genetic inputs ranged Between (0.77 - 0.18), the genotypes C1 - c and C3 - d recorded the lowest similarity or similarity percentage by 18%, while the highest similarity rate between genotype entries was between C1 - d and C1 - e with a similarity percentage of 77%. . And between the two percentages, the ratios of convergence or divergence between the plant samples within the studied population's range.

The first and second population, as well as the fourth and fifth populations, had the highest percentage of genetic affinity with varying similarity rates, while the genetic inputs of the third clan recorded similarities between other populations in varying degrees as well.

Table 3: ISSR primers are used to detect total number, polymorphic, unique bands and % of polymorphism obtain

Primers	Total no. of Bands	sdnab cihpromyloP	sdnab cihpromonoM	Polymorphism%
1 - RSSIAF - USK	100	54	46	54.00%
2 - RSSIAF - USK	150	21	129	14.00%
3 - RSSIAF - USK	100	82	18	82.00%
4 - RSSIAF - USK	50	30	20	60.00%
5 - RSSIAF - USK	75	53	22	70.70%
6 - RSSIAF - USK	150	79	71	52.70%
7 - RSSIAF - USK	100	66	34	66.00%
8 - RSSIAF - USK	50	9	41	18.00%
9 - RSSIAF - USK	50	14	36	28.00%
10 - RSSIAF - USK	100	68	32	68.00%
11 - RSSIAF - USK	50	19	31	38.00%
Totale	975	495	480	50.80%

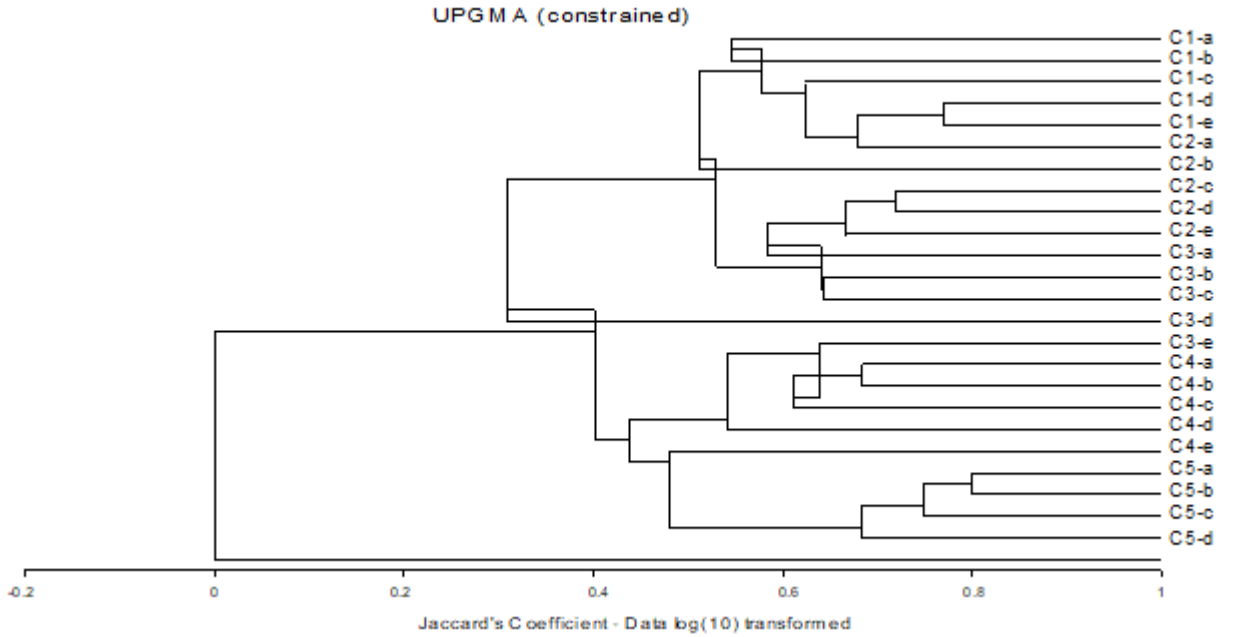

Figure 4: *C. comosum* Dendrogram using the unweighted pair group method with arithmetic average (UPGMA) based on ISSR polymorphism

Table 4: Similarity percentage matrix based on ISSR results

	C1-a	C1-b	C1-c	C1-d	C1-e	C2-a	C2-b	C2-c	C2-d	C2-e	C3-a	C3-b	C3-c	C3-d	C3-e	C4-a	C4-b	C4-c	C4-d	C4-e	C5-a	C5-b	C5-c	C5-d	C5-e
C1-a	1																								
C1-b	0.55	1.00																							
C1-c	0.68	0.56	1.00																						
C1-d	0.73	0.54	0.65	1.00																					
C1-e	0.60	0.45	0.67	0.77	1.00																				
C2-a	0.67	0.40	0.55	0.64	0.71	1.00																			
C2-b	0.50	0.41	0.52	0.45	0.57	0.63	1.00																		
C2-c	0.52	0.32	0.48	0.58	0.54	0.65	0.44	1.00																	
C2-d	0.67	0.40	0.61	0.70	0.78	0.85	0.63	0.72	1.00																
C2-e	0.61	0.44	0.62	0.59	0.55	0.67	0.58	0.67	0.67	1.00															
C3-a	0.44	0.35	0.36	0.50	0.41	0.52	0.32	0.71	0.58	0.46	1.00														
C3-b	0.52	0.34	0.48	0.57	0.63	0.75	0.55	0.76	0.75	0.70	0.56	1.00													
C3-c	0.48	0.35	0.45	0.54	0.45	0.55	0.46	0.60	0.55	0.68	0.52	0.64	1.00												
C3-d	0.27	0.20	0.18	0.31	0.20	0.39	0.23	0.35	0.33	0.38	0.32	0.37	0.50	1.00											
C3-e	0.38	0.21	0.36	0.44	0.37	0.41	0.28	0.57	0.46	0.46	0.62	0.50	0.58	0.38	1.00										
C4-a	0.36	0.24	0.35	0.38	0.36	0.45	0.31	0.48	0.40	0.39	0.59	0.48	0.44	0.25	0.67	1.00									
C4-b	0.30	0.23	0.25	0.29	0.27	0.36	0.36	0.38	0.31	0.35	0.48	0.39	0.46	0.24	0.55	0.68	1.00								
C4-c	0.32	0.21	0.36	0.39	0.37	0.41	0.28	0.50	0.41	0.46	0.48	0.50	0.46	0.32	0.70	0.59	0.63	1.00							
C4-d	0.41	0.25	0.39	0.42	0.39	0.48	0.40	0.37	0.44	0.39	0.39	0.42	0.39	0.31	0.63	0.60	0.44	0.50	1.00						
C4-e	0.42	0.21	0.33	0.44	0.40	0.40	0.24	0.43	0.40	0.39	0.40	0.44	0.28	0.28	0.47	0.38	0.32	0.56	0.44	1.00					
C5-a	0.48	0.29	0.39	0.48	0.45	0.56	0.41	0.48	0.56	0.50	0.46	0.54	0.44	0.36	0.46	0.44	0.33	0.46	0.54	0.53	1.00				
C5-b	0.48	0.29	0.44	0.43	0.40	0.45	0.36	0.37	0.45	0.44	0.35	0.43	0.39	0.30	0.40	0.39	0.28	0.40	0.60	0.53	0.80	1.00			
C5-c	0.50	0.30	0.41	0.44	0.37	0.46	0.37	0.44	0.52	0.52	0.42	0.50	0.46	0.32	0.48	0.40	0.35	0.48	0.56	0.47	0.75	0.75	1.00		
C5-d	0.42	0.30	0.45	0.48	0.45	0.50	0.52	0.43	0.50	0.56	0.36	0.53	0.40	0.22	0.41	0.44	0.35	0.46	0.59	0.39	0.70	0.63	0.73	1.00	
C5-e	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.00
	C1-a	C1-b	C1-c	C1-d	C1-e	C2-a	C2-b	C2-c	C2-d	C2-e	C3-a	C3-b	C3-c	C3-d	C3-e	C4-a	C4-b	C4-c	C4-d	C4-e	C5-a	C5-b	C5-c	C5-d	C5-e

3. Dissociation

Molecular markers play an important role in distinguishing between genetically similar genotypes, as well as in scanning genetic diversity using a large number of primers (Mosula et al., 2015). In this paper, ISSR markers have been used to efficiently determine genetic variance and genetic relationships because of their high polymorphism and usefulness in studying genetic diversity, evolution, and genetic marking (Vijayan, 2005). The level of ISSR Polymorphism was quite high among 25 genetic entries of five populations of *C. comosum* collected from different locations in Nafud Al Kabeer desert of Hail, Saudi Arabia. We obtained 975 ISSR bands using 11 primers, different primers' products differed in the number of genetic bands generated for each primer due to the difference in the complementary sites of that initiator among the studied inputs (Guasmi et al., 2006). The mean polymorphism for genetic diversity between species was 50.8%, considered high given that all accessions are from a single species of Calligonum, from multiple populations, compared to the polymorphism ratio found by Dhief et al. (2011) in their studies on three species of Calligonum plant in the Tunisian desert, which amounted to 89.06%. Variation of values in genetic diversity is normal because individuals are likely to integrate new alleles using crosses and lose alleles using genetic drift under normal conditions (Silva et al., 2012). Through the dendrogram there is a genetic relationship within the clustered marker system, the genotypes of the genetic inputs were grouped, and enough genetic variation was found between them. The similarity ratio differed between high and low was found in some of the analyzed individuals as indicated by the values of the similarity matrix. It is possible that a species had different ancestors or was influenced by alternative evolutionary processes It is also possible that this genotype was driven by greater numbers Human activity and human actions such as cutting or protection may also have affected population sizes and thus the genetic diversity of some species (Silva and Gardner, 2015).

4. Conclusion

We conclude from the above that ISSR markers provided good information for the genetic diversity available in *C. comosum* inputs from the northern Hail region of Saudi Arabia, and the results of this study will help to understand genetic variation and evolutionary dynamics in *C. comosum* and expand the genetic base for breeding wild species. That additional selection of inputs in each species to assess genetic diversity leads to more useful and reliable results. Genetic conservation programs in arid environments rely on molecular methods for diversity assessments. DNA - based molecular profiling will aid in the conservation and protection of species from genetic erosion

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