GENETIC POLYMORPHISM IN THREE POPULATIONS OF THE GREY-LEAVED CORDIA (*CORDIA SINENSIS* LAM.) FROM KHARGA OASIS, SOUTHWEST EGYPT

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Abstract. The present study aimed at determining the level of genetic polymorphism within and among three grey-leaved cordia (*Cordia sinensis*) populations in southwest Egypt. It also aimed at giving a preliminary genetic relationship between populations widely distributed across the different localities in the study area. Twenty-four genotypes representing three populations (Kharga, Dabadib, and Paris areas) were collected from Kharga Oasis to evaluate their genetic variability using 15 random amplified polymorphic-DNA (RAPD) primers. Twelve genotypes from Kharga area were moderately polymorphic (66.4%), while the desert habitats of north and south of Kharga area (Dabadib and Paris oasis), showed lower polymorphism (44.3 and 49.5% respectively). Jaccard's similarity index indicated that Dabadib and Paris populations were related to each other with the highest similarity index (0.69). The dendrogram generated by the agglomerative clustering technique produced three main clusters: two of them for Kharga genotypes and the 3rd for Dabadib and Paris genotypes indicating the close relations between these populations. The genetic diversity indicated that Paris populations had the highest Shannon and Simpson indices (5.3 and 242.4, respectively), while Kharga had the lowest (4.8 and 198.4). The higher polymorphism in Kharga area than in Dabadib and Paris may suggest the species confinements into more favorable and very stable habitats. The current findings may help in the conservation of such medicinally and economically important species for future use by local people in such harsh and remote habitats. However, the study showed the need for *in-* and *ex-situ* conservation of *C. sinensis* for plant breeding programs and the future use in medicine, fodder and wood industry. The polymorphic primers identified here will be useful in identifying evolutionary relationship and taxonomic position to understand the phylogeny of *the genus Cordia belonging to family Boraginaceae.*
Keywords: *grey-leaved cordia, Boraginaceae, genetic diversity, RAPD, medicinal plant, antimalarial*

Introduction

The grey-leaved cordia (*Cordia sinensis* Lam.), belonging to the family Boraginaceae, is a tropical tree distributed mainly in Africa and Asia (Orwa et al., 2009). The species extends to south Egypt in the moist ground (Oases) and open desert scrub of Gebel Elba (Boulos, 2000; Barroso and Oliveira, 2009). This species is only known from gardens in south-western Egypt, and it seems to be limited to cultivated or naturalized stands in Yemen and Palestine (Galal et al., 2024a). The plant is self-pollinating and the seedlings are produced using seeds (Ndung'u and Kimiti, 2017). It grows in clay, clay-sand, or other alluvial soils at elevations up to 1240 m above sea level (Abdel Kawey, 2016). *C. sinensis* can also thrive in arid regions on steep hillsides, in limestone cliff crevices, and along wadis (Warfa, 1988). In the IUCN Red List of Threatened Species, it was listed as Least Concern in 2020 (Shaltout and Bedair, 2022). In popular medicine *Cordia* species is used to treat stomach disorders, chest pains (Richard et al., 2010), and malaria (Orwa et

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al., 2009). The leaf paste is applied on an open wound and also useful in inflammation of legs (Zala et al., 2012). *C. sinensis* possesses anti-inflammatory, antidiabetic, antioxidant, antileprotic, antidiarrheal, and antiseptic activities and reduces burning sensation of urinary tract (Musayeib et al., 2011; Marini et al., 2018; Chen et al., 2023). Moreover, *C. sinensis* is a popular indigenous multipurpose tree species that is useful for fuel wood, timber, fruits, and fodder (Galal et al., 2024b).

Plant growth occurs as a result of the mutual interaction of genetic structure and environmental conditions (Owino et al., 2021). Morphological, anatomical, and phenotypic characteristics of plants come up as a result of the interaction of genetic structure and environmental conditions (Yucedag et al., 2019). Recently, chromosome-scale genomes have been reported in numerous plant species, which provide insights into their genetic basis and population genetic structure (Chen et al., 2023). Although *Cordia* spp. are both medicinally and economically important (Galal et al., 2024a), few studies have been carried out to study polymorphism in the different populations at the genetic level. Randomly amplified polymorphic DNA (RAPD) markers are widely used for polymorphism analysis and classifying genotypes in clusters according to genetic relationships (El-Bakry et al., 2014). Genetic variation in *Cordia* species were studied using molecular marker techniques; RAPD markers was used for molecular analysis of *C. Dichotoma* (Nandedkar and Mulani, 2016), while RAPD and ISSR markers were applied in *C. myxa* (Sivalingam et al., 2012; Nikkhah et al., 2022), respectively. Besides, amplified fragment length polymorphisms (AFLPs) and chloroplast microsatellite markers were investigated in *C. africana* (Derero et al., 2011), *C. verbenacea* (Figueira et al., 2010), *C. bifurcata* (Spoon and Kesseli, 2008), and *C. alliodora* (Chase et al., 1995).

In the tropical regions, there is a need to undertake landscape restoration to improve degraded former forest lands (König et al., 2023). Combined with climate change, this poses huge challenges to both the amount and quality of the plant material used for this restoration (Ousmael et al., 2024). Consequently, there is a need to conserve and improve trees of a multitude of species. *C. sinensis* varies considerably in size, dentations and indumentums of the leaves; most variations seem to be environmentally conditioned, but there are also some regional trends (Galal et al., 2024a). There is a continuing decline in the number of locations and mature individuals in the wild, thus this is the reason some researchers consider the species extinct in the wild (Ammar et al., 2020). The major threats to the plant are over-exploitation by local people who harvest the species to make valued walking sticks sold commercially at Aswan (Shaltout and Bedair, 2022). Therefore, there is urgent need for in- and ex-situ conservation of *C. sinensis* for plant breeding programs and the future use in medicine, fodder and wood industry.

The present study aimed to evaluate the genetic variability of three *C. sinensis* populations in southwest Egypt to determine the level of genetic polymorphism within and among these populations. According to our knowledge, this study may be the first one to assess the genetic variation within and among populations of *C. Sinensis*. This may help in the conservation of these medicinally and economically important species in harsh and remote habitats, and enable their future use by local people.

Materials and methods

The study area (Kharga Oasis) lies about 200 km west of the Nile between latitudes 24° 30′ N - 26° 00′ N and longitudes 30 º 27′ E - 30º 47 E (*Fig. 1a*). The extreme length of Kharga depression is about 200 km, while its width varies from 20 to 80 km with an

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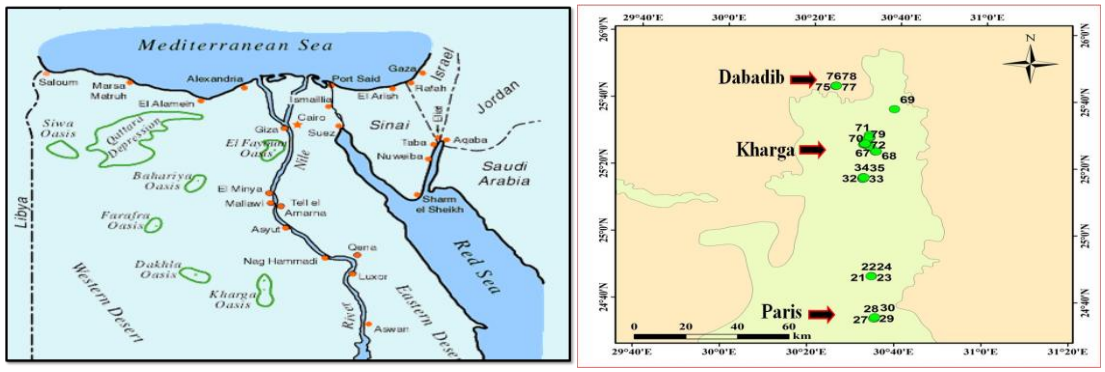
area of 7200 km^2. Three different localities; Kharga, Dabadib, and Paris were selected (*Fig. 1b*). Kharga are characterized with some habitats including fallow lands, road sides, ruderal areas, cultivated areas, desert and sand dunes; Dabadib area characterized with sand dunes and desert habitats; and Paris Oasis represented by cultivated areas reaching edges of the desert and sand dunes. These ecological variations may affect the distribution, abundance and growth performance of the study species. The prevailing climate indicated that the study area lies in a dry rainless part of the Great Sahara with average annual rainfall of 0.0042 mm year^{-1}, while the mean annual temperature was 25.5°C. Moreover, the mean annual relative humidity was 35.9%, while the annual mean wind speed was 5.5 km h^{-1} (NASA–POWER, 2015).



(a) (b)

Figure 1. *Location map of Egypt showing (a) Kharga Oasis, (b) the three localities (Dabadib, Kharga, and Paris) under study*

Twenty-four individuals collected during spring season from the different habitats of the study area were chosen for genetic analysis. They were distributed as 12 individuals from Kharga area, and six individuals from both Paris and Dabadib. Three top leaves from each individual were collected, dried at room temperature, and then stored in paper bags till DNA extraction.

Random amplified polymorphic DNA (RAPD) analysis

A preliminary experiment on four randomly selected genotype was carried out to select most suitable primers for identification. Twenty-five RAPD primers were screened for repeatability, scorability, and their ability to distinguish between genotype (*Table 1*). Then, RAPD analysis was performed to establish fingerprints for 24 individuals collected from the three different localities (Dabadib, Kharga and Paris) using 15 different RAPD Operon primers (OP A1, A3, A7, A9, A10, B3, B5, B7, B8, D2, D3, E1, E3, E8 and F9) (*Table 2*), where they revealed reproducible polymorphic patterns and were used for further analysis.

DNA extraction

DNA from three dried leaf samples collected from each locality was extracted using genomic DNA extraction kits (GE Healthcare, illustraTM Nucleon Phytopure Genomic DNA Extraction Kits, Codes RPN 8510, UK). The purity and concentration of each sample was measured. 0.01 g dry weight of leaf tissue was ground in liquid nitrogen to yield a free-

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flowing powder. The powder was transferred using a chilled spatula, to 2.0 ml cooled Eppendorf tube. Then 300 µl of buffer A were added and dissolved by mixing thoroughly with a spatula. After that 900 µl of buffer B were added and the tube was inverted several times until a homogeneous mixture was obtained. Then, 100 µl of SDS were added and the tube was inverted several times until a homogeneous mixture was obtained.

Table 1. *Twenty-five RAPD primers and total amplified fragments (TAF) selected for the preliminary study*

Primers	TAF	Primers	TAF
OPA1	5.9	OPD2	6.4
OPA3	13.8	OPD3	8.3
OPA7	6.6	OPD5	3.8
OPA9	6.4	OPD7	4.6
OPA10	7.4	OPE1	5.6
OPB3	5.5	OPE2	3.5
OPB4	3.6	OPE3	7.4
OPB5	7.5	OPE5	2.1
OPB7	9.9	OPE8	6.5
OPB8	6.5	OPE9	2.3
OPC1	4.4	OPF1	4.5
OPC7	2.7	OPF3	3.0
		OPF9	6.1

Table 2. *The nucleotide sequence of the chosen 15 RAPD Operon primers*

Primer code	Nucleotide sequence 5′→3
A1	CAGGCCCTTC
A3	AGTCAGCCAC
A7	GAAACGGGTG
A9	GGGTAACGCC
A10	GTGATCGCAG
B3	CATCCCCCTG
B5	TGCGCCCTTC
B7	GGTGACGCAG
B8	GTCCACACGG
D2	GGACCCAACC
D3	GTCGCCGTCA
E1	CCCAAGGTCC
E3	CCAGATGCAC
E8	TCACCACGGT
F9	CCAAGCTTCC

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Agarose gel preparation

TBE buffer (5x) was prepared by dissolving 54 g Tris base and 27.5 g boric acid in 20 ml 0.5 M EDTA (pH = 8.0) and volume was completed to 1 L using distilled water. Working solution 0.5 x TBE buffer was made by 1:10 dilutions from 5x TBE buffer. One gram of agarose in 100 ml of 0.5x TBE buffer was melted by boiling the mixture till the agarose dissolved. Cooling for a couple of minutes then 2.5 µl of ethidium bromide was added with stirring to mix. The gel was casted using a supplied tray and comb and left for a minimum of 30 min at room temperature to set on a flat surface 5.0 µl of sample and 5.0 µl of H_2O with 1.0 µl of 6x loading buffer (bromophenol blue) were loaded into well.

Polymerase chain reaction (PCR)

For each reaction, 50 ng of DNA and 0.5 µM primer per 25 µl reaction were added to a tube containing Taq DNA polymerase. Tubes caps were snapped firmly to ensure tight fit then tubes contents were mixed by gently flicking with a finger, vortexes gently and then centrifuged for a few seconds to bring the components to the bottom of the tube. Reactions were performed as described by William et al. (1990) with some modifications 15 Operon primers from Bio Basic Inc., Canada. A DNA thermal cycler (Mastercycler personal from Eppendorf, Germany), was programmed as follows: 1 cycle of 94°C for 5 min (initial strand separation), followed by 50 cycles for 1 min at 94°C (denaturation), 1 min at 36°C (annealing), 2 min at 72°C (elongation), 1 cycle of 10 min at 72°C (final extension) and 4°C for 30 min.

DNA agrose gel electrophoresis

After amplification, PCR products were separated by gel electrophoresis (Wealtec Crop Model: GES, Taiwan) in 1.2 % agarose gel (Lonza, Seakem LE Agarose, USA), stained with ethidium bromide and a 1 Kb DNA ladder (Gene Dire X- USA) used. The run was performed at 100 V for 5 min. then at 85 V. the products were visualized and photographed under UV transilluminator (Wealtec Mode MD-20, 312 nm, Taiwan).

Data analysis

Gel images banding patterns of RAPD were analyzed using Quantity 1 software program (BioRad laboratories, Hercules, California). Reproducible bands were fed to the computer as 1 and 0 for the presence and absence of bands, respectively. The generated binary data was used to estimate the levels of polymorphism by dividing the polymorphic bands by the total number of scored bands. The genotype and allelic frequency data were used to compute the genetic diversity indices, i.e., (1) Shannon's information index and Simpson index expressed as $\hat{H} = -\Sigma^{s}_{i=1}$ Pi (log Pi), D = 1/C and $C = \Sigma^{s}_{i=1}$ $(Pi)^2$, respectively where S is the total number of alleles and P is the proportion of polymorphic loci/total loci. The binary data matrix was used to calculate Jaccard's similarity coefficient between pairs of accessions using CAP (Community Analysis Package) software program as mentioned in El-Bakry et al. (2014). These distance's coefficients were used to construct dendrogram using the clustering employing the sequential agglomerative single linkage for determining the genetic diversity and relationships among the accessions (localities).

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Results

The concentration and purity of total extracted genomic DNA from dried leaf samples collected from three localities (Kharga, Dabadib and Paris) were measured. The values of DNA concentration and purity ranged from 1000 to 3360 ug/ml and from 1.4 to 1.9, respectively. The genetic data for each of the three populations of *C. Sinensis* is given in *Table 3* and *Figure 2*. In Kharga populations, a total amplified fragments of 223 bands were scored among 12 studied genotypes. Besides, a total of 148 polymorphic bands with 66.4% polymorphism were obtained with the highest polymorphism (76.9%) in OPA10 and the lowest (50%) in OPA9 primers. Moreover, 67 monomorphic bands with 30.5% monomorphism and 8 unique bands (3.1%) were recorded. Whereas, Dabadib and Paris populations recorded 206 TAF bands for each among the six genotypes with a total of 93 and 103 polymorphic bands exhibiting 44.3 and 49.5% polymorphism, respectively. The highest polymorphism noticed (62.5 and 66.7%) with Primers OPB5 and OPA10 for Dabadib and Paris populations, respectively.

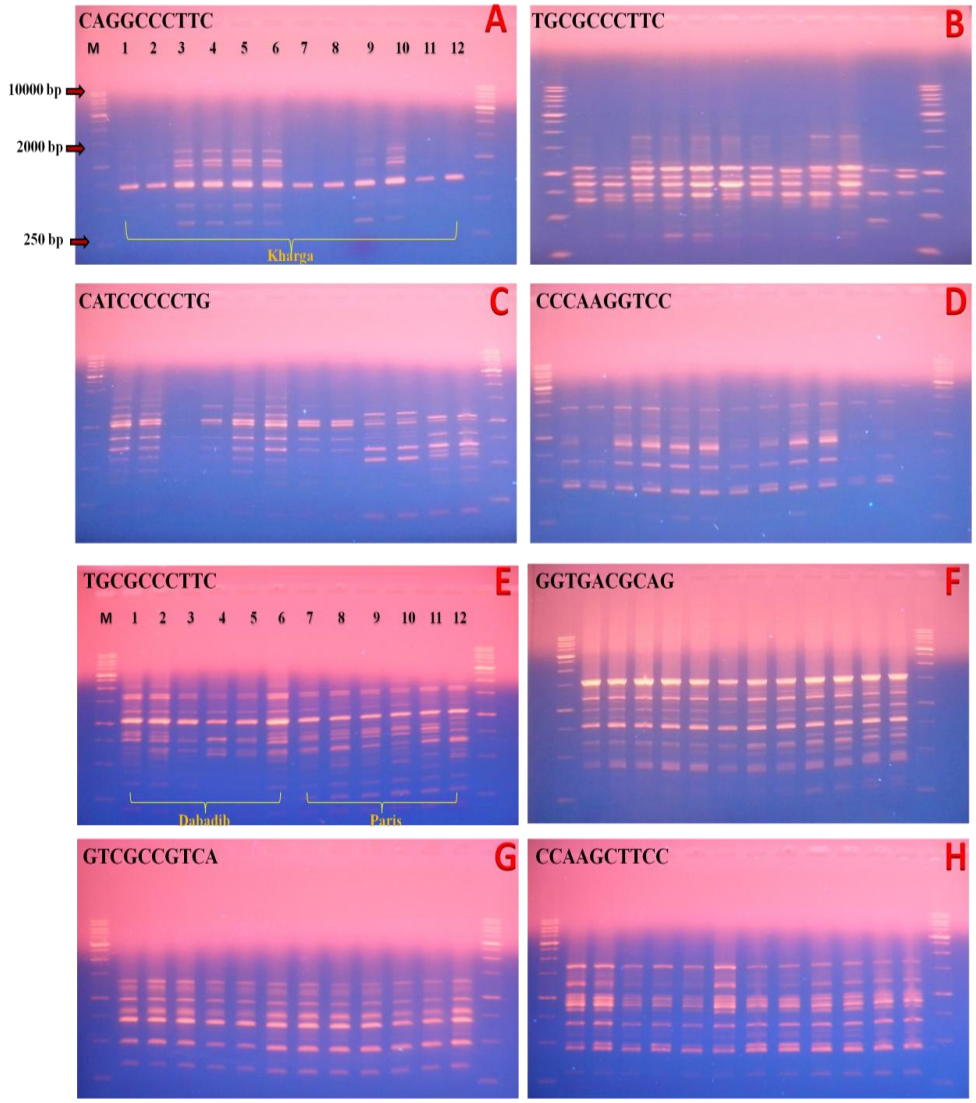

Figure 2. *RAPD banding patterns of Cordia sinensis from the three localities using M = 1 Kb ladder. A-D: Kharga genotypes (1:12), E-H: Dabadib genotypes (1:6), and Paris genotypes (7:12)*

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Table 3. *Polymorphism in C. sinensis using 15 random primers showing total amplified fragments (TAF), polymorphic, monomorphic and unique bands*

Primer code	Kharga							Dabadib							Paris						
	TAF		Polymorphic		Monomorphic		Unique	TAF		Polymorphic		Monomorphic		Unique	TAF		Polymorphic		Monomorphic		Unique	
		Bands	%	Bands	%	Bands	%		Bands	%	Bands	%	Bands	%		Bands	%	Bands	%	Bands	%
A1	8	6	75.0	2	25.0	0	0	9	3	33.3	6	66.7	0	0.0	9	3	33.3	5	55.6	1	11.1
A3	17	12	70.6	4	23.5	1	5.6	14	8	57.1	5	35.7	1	7.1	14	8	57.1	6	42.9	0	0.0
A7	23	14	60.9	7	30.4	2	8.7	23	12	52.2	9	39.1	2	8.7	23	9	39.1	11	47.8	3	13.1
A9	8	4	50.0	4	50.0	0	0	6	2	33.3	3	50.0	1	16.6	6	3	50.0	3	50.0	0	0.0
A10	13	10	76.9	2	15.4	1	7.7	15	6	40.0	9	60.0	0	0.0	15	10	66.7	5	33.3	0	0.0
B3	15	10	66.7	4	26.7	1	6.7	15	8	53.3	7	46.6	0	0.0	15	8	53.3	5	33.3	2	13.3
B5	16	12	75.0	4	25.0	0	0	16	10	62.5	5	31.3	1	10.0	16	8	50.0	8	50.0	0	0.0
B7	20	13	65.0	6	30.0	1	5	18	7	38.9	11	61.1	0	0.0	18	11	61.1	7	38.9	0	0.0
B8	17	10	58.8	6	35.3	1	5.9	15	8	53.3	5	33.3	2	13.3	15	7	46.7	6	40.0	2	13.3
D2	11	7	63.6	4	36.4	0	0	11	4	36.4	7	63.6	0	0.0	11	4	36.4	6	54.5	1	9.1
D3	14	8	57.1	5	35.7	1	7.1	13	5	38.5	4	30.8	4	30.8	13	8	61.5	5	38.5	0	0.0
E1	16	11	68.8	5	31.3	0	0	11	6	54.5	5	45.5	0	0.0	11	4	36.4	6	54.5	1	9.1
E3	11	8	72.7	3	27.3	0	0	11	5	45.5	5	45.5	1	9.1	11	5	45.5	6	54.5	0	0.0
E8	17	11	64.7	6	35.3	0	0	13	7	53.8	5	38.5	1	7.8	13	8	61.5	5	38.5	0	0.0
F9	17	12	70.6	5	29.4	0	0	16	2	12.5	12	75.0	2	12.5	16	7	43.8	9	56.3	0	0.0
Total	**223**	**148**	**66.4**	**67**	**30.5**	**8**	**3.1**	**206**	**93**	**44.3**	**98**	**48.2**	**15**	**7.7**	**206**	**103**	**49.5**	**93**	**45.9**	**10**	**4.6**

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Furthermore, the specific markers (unique bands) with their molecular size for the three populations were presented in *Table 4*. The 223 amplified fragments in Kharga populations have 8 positive specific markers with the highest number (2) obtained by OP A7. On the other side, among 206 amplified fragments in Dabadib and Paris, 15 and 10, respectively were positive specific markers with the highest number (4 and 3) obtained by OP D3 and OP A7, respectively.

Table 4. *Number and molecular size in bp of the amplified unique fragment DNA bands generated by 15 random primers in the three populations of Cordia sinensis*

Primer code	Approximate band size (bp)	Kharga		Dabadib		Paris	
		No.	Size (bp)	No.	Size (bp)	No.	Size (bp)
A1	1460-354	0	0	0	0	1	1445
A3	2523-385	1	2523	1	1947	0	0
A7	3611-200	2	3611-2611	2	1345-460	3	2541-2611-1574
A9	1666-390	0	0	1	501	0	0
A10	1791-271	1	1791	0	0	0	0
B3	2025-230	1	2025	0	0	2	1711-1308
B5	1888-288	0	0	1	407	0	0
B7	2716-143	1	2716	0	0	0	0
B8	2311-410	1	2311	2	1900-1631	2	1900-1631
D2	1610-297	0	0	0	0	1	1204
D3	2120-246	1	2120	4	1784-1393-1234-900	0	0
E1	2500-234	0	0	0	0	1	447
E3	1923-567	0	0	1	777	0	0
E8	2272-277	0	0	1	1535	0	0
F9	2081-253	0	0	2	1812-511	0	0

The genetic diversity indicated that Paris populations had the highest marker index (*Table 5*); Shannon index and Simpson index (5.3 and 242.4, respectively), followed by Dabadib populations (5.2 and 229.1), while Kharga had the lowest (4.8 and 198.4).

Table 5. *Genetic diversity indices of Cordia sinensis genotypes within the studied populations*

Diversity index	Population		
	Kharga	Dabadib	Paris
Shannon index	4.8	5.2	5.3
Simpson index	198.4	229.1	242.4

Jaccard's similarity index within populations of the three localities showed higher similarity value from 0.62 to 0.80 within Paris genotypes, from 0.57 to 0.73 within Dabadib genotypes, and from 0.42 to 0.73 within Kharga genotypes (*Table 6*). Moreover, similarity index among populations showed higher similarity between Dabadib and Paris (0.57-0.75), while the lowest overall mean from 0.42 to 0.62 was obtained among Kharga and Dabadib genotypes.

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Table 6. *Similarity indices among Kharga (K), Dabadib (D), and Paris (P) populations estimated by RAPD analysis*

	K1	K5	K9	K12	K15	K17	K20	K22	K31	K33	K41	K49	D52	D53	D54	D55	D56	D57	P60	P61	P62	P63	P64
K5	0.65																						
K9	0.60	0.70																					
K12	0.60	0.66	0.72																				
K15	0.59	0.61	0.68	0.73																			
K17	0.62	0.63	0.63	0.69	0.69																		
K20	0.62	0.52	0.62	0.62	0.63	0.66																	
K22	0.54	0.56	0.58	0.58	0.57	0.60	0.63																
K31	0.57	0.58	0.65	0.62	0.58	0.58	0.62	0.64															
K33	0.55	0.54	0.57	0.58	0.57	0.53	0.55	0.55	0.68														
K41	0.51	0.51	0.52	0.50	0.49	0.52	0.57	0.55	0.58	0.57													
K49	0.55	0.51	0.50	0.50	0.52	0.50	0.58	0.52	0.59	0.55	0.66												
D52	0.51	0.48	0.51	0.47	0.53	0.57	0.49	0.47	0.53	0.50	0.45	0.42											
D53	0.54	0.57	0.54	0.52	0.53	0.61	0.52	0.48	0.53	0.48	0.44	0.45	0.70										
D54	0.47	0.48	0.54	0.52	0.52	0.56	0.48	0.45	0.51	0.52	0.46	0.42	0.68	0.68									
D55	0.47	0.52	0.50	0.54	0.51	0.60	0.50	0.45	0.50	0.50	0.42	0.43	0.68	0.70	0.73								
D56	0.51	0.52	0.55	0.59	0.62	0.62	0.56	0.52	0.56	0.54	0.51	0.47	0.62	0.63	0.67	0.67							
D57	0.50	0.51	0.56	0.57	0.56	0.66	0.59	0.50	0.55	0.54	0.46	0.43	0.65	0.64	0.64	0.68	0.75						
P60	0.48	0.48	0.54	0.57	0.56	0.59	0.57	0.53	0.56	0.52	0.48	0.45	0.57	0.61	0.60	0.60	0.67	0.66					
P61	0.54	0.55	0.55	0.57	0.57	0.62	0.59	0.55	0.62	0.55	0.52	0.50	0.64	0.61	0.59	0.61	0.67	0.65	0.72				
P62	0.51	0.48	0.54	0.56	0.57	0.63	0.59	0.54	0.59	0.58	0.51	0.47	0.61	0.58	0.60	0.61	0.70	0.68	0.68	0.72			
P63	0.52	0.51	0.57	0.54	0.56	0.59	0.54	0.50	0.59	0.59	0.53	0.47	0.60	0.62	0.63	0.60	0.65	0.63	0.63	0.68	0.70		
P64	0.52	0.51	0.54	0.53	0.56	0.59	0.53	0.48	0.58	0.54	0.55	0.48	0.60	0.62	0.64	0.58	0.62	0.62	0.62	0.69	0.70	0.72	
P65	0.49	0.53	0.58	0.54	0.57	0.60	0.57	0.51	0.59	0.55	0.53	0.51	0.62	0.63	0.61	0.60	0.64	0.67	0.65	0.73	0.69	0.72	0.80

The dendrogram generated by the agglomerative clustering technique using Community Analysis Package (CAP) software with the Euclidean distance among the three populations produced three main clusters (*Fig. 3*): A and B for Kharga genotypes and C for Dabadib and Paris genotypes indicating the closer relation between the latter two populations than Kharga genotypes.

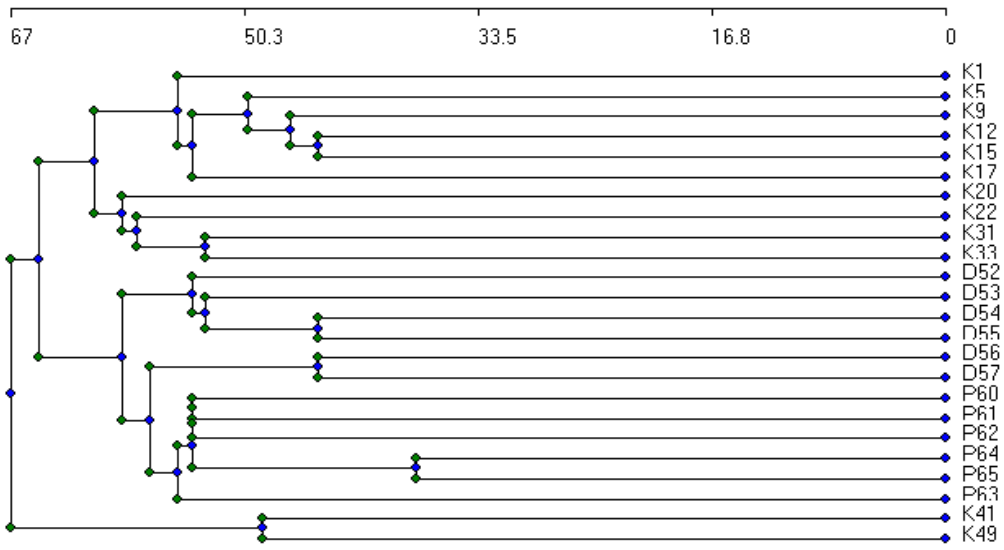

Figure 3. *Genetic relationships within and among three Cordia sinensis populations by CAP analysis (single linkage); K: Kharga genotypes, D: Dabadib genotypes, and P: Paris genotypes*

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Discussion

Genetic variation is critical to the long-term survival of a species because it allows for the development of required adaptations to deal with alterations in the ecosystem (Nikkhah et al., 2022). According to Freeland et al. (2011), the genetic diversity and polymorphism of the *C. sinensis* populations were low to moderate. There is a strong correlation between the extent of genetic variation within a species and the manner of reproduction; the more open pollination/cross-breeding occurs, the more genetic variability is seen in the taxon under study. Given that *C. sinensis* is mostly a self-pollinating species (Ndung'u and Kimiti, 2017), it is possible that the minimal degree of genetic diversity among populations is due to the intimate pattern of reproduction in this taxon. Simila results were reported by Nikkhah et al. (2022) on *C. myxa*.

Polymorphic primers are useful in identifying evolutionary relationship and taxonomic position to understand the phylogeny of the genus *Cordia*, sequence information with morphological, ecological and biogeographically data (Gottschling et al., 2005). Fifteen primers revealed reproducible polymorphic patterns were used for genetic analysis of *C. sinensis*. In the current study, the DNA concentration and purity in *C. sinensis* ranged from 1000 to 3360 ug/ml and from 1.4 to 1.9, respectively. In similar results on *Heliotropium bacciferum* and *Arnebia decumbens* (Boraginaceae), Alarkwazi et al. (2021) reported values of DNA concentration (110 and 70 ng/ml) and purity (1.75 and 1.70), respectively. Additionally, the RAPD markers were used for molecular evaluation of the genetic polymorphism in *C. dichotoma* and recorded 67.86% polymorphism (Nikkhah et al., 2022), which approximated our recorded value for *C. sinensis*. Also, there was a range of genetic variation shown by Jaccard's co-efficient, from 0.48 to 0.86 (Nikkhah et al., 2022), which coincided with our findings.

Genetic diversity has been found to vary directly with population size (Travis et al., 1996). The present study revealed moderate level of polymorphism (66.4, 44.3, and 49.5%) with a total number of amplified fragments (223, 206, and 206) and positive specific markers (8, 15, and 10) within the populations of Kharga area, Dabadib and Paris, respectively. Additionally, the similarity index was higher (0.57-0.75) between Dabadib (North of Kharga) and Paris (South of Kharga) populations. These results are consistent with the results reported by Sivalingam et al. (2012), who found that the average polymorphism revealed by RAPD marker among 22 accessions of *C. myxa* from Indian hot arid region was 69.8% with an average polymorphic information content of 0.43. Whereas they reported genetic diversity, revealed by Jaccard's co-efficient, between 0.44 and 0.94. Moreover, Derero et al. (2011) demonstrated that, the analyses of the amplified fragment length polymorphisms (AFLP) data revealed high diversity in all investigated populations of *C. africana*. The percentage of polymorphic loci (PPL) ranged from 62.2% to 92.2% and Nei's gene diversity from 0.22 to 0.32 within the populations. The mean PPL and the mean diversity within populations were 85.7% and 0.29, respectively

The results of the present study may exhibit phytogeographical significance, since the target species extends north up till Jordan and south till South Africa, and remnants of the species fruits were found in the archeological site of Qena south of Egypt (Fahmy, 2005). The higher polymorphism was noticed in Kharga, where habitats are more variable and versatile than in Dabadib and Paris (mostly desert habitats). It is possible that the species was widely distributed in the past and has been recently localized in specific habitats with changing climatic conditions. It is expected that with today's trend of global warming and climatic changes, the species will be more

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threatened with present hyper-arid conditions. This will suggest that the conservation of *C. sinensis* both in- and ex-situ should be carried out for the preservation of valuable germplasm of the wild species of both medicinal and economic importance. According to van Chiocchio et al. (2024), populations with higher levels of genetic diversity have proved to be the less vulnerable to the detrimental consequences of genetic drift and, at the same time, the more likely to evolve traits increasing population-level fitness in response to novel environmental conditions (Forester et al., 2022). Furthermore, genetic diversity is crucial for a healthy population as it maintains different genes that could lead to resistance to pests, diseases, or other stress conditions; besides, it also enables individuals to adapt to various biotic and abiotic stresses, particularly in arid and semi-arid regions (Salgotra and Chauhan, 2023). According to Thomas et al. (2014), high genetic diversity can help ensure survival of sufficient numbers of trees that are planted in a degraded ecosystem to increase their chances of establishment and survival at the planting sites.

Conclusion

The obtained results dealt with the genetic variability and relationships within and among three populations (Kharga, Dabadib and Paris) of *C. sinensis* genotype. The highest polymorphism noticed with Primers OPB5 and OPA10 for Dabadib and Paris populations, respectively. Jaccard's similarity index indicated that Dabadib and Paris populations were related to each other. The genetic diversity indicated that Paris populations had the highest Shannon and Simpson indices, while Kharga had the lowest. The higher polymorphism in Kharga area than in Dabadib and Paris may suggest the species confinements into more favorable and very stable habitats. The polymorphic primers identified here will be useful in identifying evolutionary relationship and taxonomic position to understand the phylogeny of the genus *Cordia* belonging to family Boraginaceae, and the sequence information with morphological, ecological and biogeographical data. Furthermore, genetic variability among C. sinensis populations can help in breeding programs and ecological restoration projects

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Conflict of interests. The authors declare that they have no competing interests.

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