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Genetic variation of arta populations (*Calligonum polygonoides* subsp. *comosum*) in Egypt: Genepools for biodiversity and afforestation

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Abstract: Genetic diversity manipulates a prime and vital role in the sustainable use of genetic resources. The data highlighted more insights into the genetic diversity of the arta plant (*Calligonum polygonoides* subsp. *comosum*) populations collected from three localities, Qalabshu (QQ), Mutubas (MM) and Gamasa (GG), in Egypt as gene pool for biodiversity conservation and afforestation. Random amplified polymorphic DNA (RAPD) markers investigated the population pattern and structure. A total of 129-amplicons oscillated from 145 to 1505 bp and a total of 19-amplicons were specific markers with an average of nine bands for each population. The Shannon index (*I*) scored at an average of 0.3. The diversity (*h*) oscillated from 0.11 to 0.25. The similarity matrices based on Jaccard coefficient recorded positive values. A higher correlation (r = 0.83) was between the combined Qalabshu (*QQ)* and Mutubas (*MM)* matrices using the Mantel test with 1,000 permutations. This species has higher adaptability for their regions. This gene pool is a valuable reservoir for enriching genetic diversity and provides basal information for the biodiversity conservation of a dominant species. The dominant species can be utilised in afforestation in the same region or another region which has the same environmental conditions.

Keywords: afforestation, biodiversity conservation, *Calligonum polygonoides*, genetic diversity

INTRODUCTION

Genetic diversity is a basic of the evolutionary potential within and between species populations [Ma *et al.* 2008; Pervaiz *et al.* 2020; Prieto *et al.* 2015; Wu 2019]. The estimating of the level and pattern of genetic diversity of natural populations provides more insights into the evolutionary history of species, e.g., habitat fragmentation, shifts in distribution and population isolation, and the basis of improvement programs [Blanchet *et al.* 2020; Raffard *et al.* 2019; Ueno *et al.* 2000].

Calligonum polygonoides L. subsp. *comosum* L'Hér and *C. polygonoides* L. subsp. *polygonoides* L. belong to the family Polygonaceae [Purohit, Kumar 2020; Sanchez *et al.* 2011; Soskov 2011; The Plant List 2013] and locate commonly in Egypt [Boulos 2009]. Boulos [1995; 2009] stated *comosum* species are very distributed frequently in Oases, Mediterranean coastal strip, all desert regions, Red Sea Coastal Region, Gebel Elba, and Sinai. *Polygonoides* species are very rare and disseminated in El-Tih and North of Wadi Tumilat region in Egypt. The current events in Egypt are obstructed and disserved in this study to collect and conserve both species of arta plant. Additionally, no efficient study had conducted on populations of arta plant. This species is woody perennial xerophytic shrubs growing on dry sandy soils and dunes, dominant in desert flora, and slow-growing condensed leaves with woody branches photosynthesise instead, usually 4–10 feet high [Boulos 1999; 2009; Sanchez *et al.* 2011; Soskov 2011]. It is capable of growing under adverse conditions of soil and moisture. Its root is suckers and easily propagates by cutting and layering [Maryamgul *et al.* 2012; Sanchez *et al.* 2011; Soskov 2011; Tadevosyan 2001]. *Calligonum*, with other dominant species, can stabilise the dunes by a dense network of roots [Dhief *et al.* 2011; Soskov 2011]. It has multi-uses as food [Bewal

et al. 2008; Zaher *et al.* 2020], fuel [Singh, Wadhwani 1996], therapeutic activity [Vyas *et al.* 2012; Zaher *et al.* 2020], and fodder [Goyal, Sharma (eds.) 2006; Pervaiz *et al.* 2020]. The aqueous paste of the plant is an antidote against the heavy doses of opium and the poisonous effects of certain harmful plants [Pervaiz *et al.* 2020; Singh, Wadhwani 1996]. Various human activities can lead to dimmish populations at a dangerous rate that affects *Calligonum* diversity and its genetic structure. No study of the genetic diversity of the *Calligonum* populations had done in Egypt.

Calligonum species can utilise through various investigations, such as morphological description [Boulos 1995; 2009; Purohit, Kumar 2020], chemical and biochemical analysis [Pervaiz *et al.* 2020; Samejo *et al.* 2013; Tao, Ren 2004; Vyas *et al.* 2012], molecular markers [Dhief *et al.* 2011; Ren, Tao 2002; Soskov 2011; Vyas *et al.* 2012]. Molecular markers proved a valuable characterisation of the genetic diversity in a species. Various molecular markers might reveal different classes of polymorphism [Kim *et al.* 2019; Powell *et al.* 1996; Russell *et al.* 1997; Sun, Zhang 2012; Wu *et al.* 2004; Xiang *et al.* 2015]. Various molecular markers had utilised for detecting the *Calligonum* genetic diversity [Dhief *et al.* 2011; Ren, Tao 2002; Song *et al.* 2020; Soskov 2011; Tao, Ren 2004; Vyas *et al.* 2012].

The novelty in biodiversity comes significantly from fast and ongoing genetic variations created in ecosystems everywhere. Global novelty assessment is based on biotic and abiotic factors. Interestingly, the places that are most novel are often not the places where absolute changes are largest; highlighting that novelty is inherently different from change. Increasing novelty presents opportunities for biodiversity conservation that is necessary along the entire continuing novelty by multiplying efforts to conserve places where novelty is low. Meeting the challenges of the novelty will require advances in the multi-disciplines and creative biodiversity conservation approaches [Radeloff *et al.* 2015]. Arising several issues need to monitor located commonly in Egypt. The historical gene pool of arta plant genetic diversity is in how retreating the biodiversity conservation and afforestation. Genetic structure within arta plant propagated and forecasted is forming mixed afforestation in the future. Isolated or intermating gene pools of species are structured. Revealing these properties will improve understanding of species ecology and support the natural integration of the arta plant into located commonly in Egypt.

Three natural populations of *C. polygonoides* cumulated from Mutubas, Qalabshu, and Gamasa. These populations investigated the genetic diversity using RAPD (random amplified polymorphic DNA) markers. The emanated data provide more insights into the level and pattern of genetic diversity of the arta population within species in Egypt.

MATERIALS AND METHODS

COLLECTION AND SAMPLING OF *C. POLYGONOIDES*

Populations of *C. polygonoides* L. subsp. *comosum* L'Hér were collected from dominant localities as shown in Table 1 and Figure 1 and deposited at National Gene Bank (NGB), Agricultural Research Centre (ARC), Egypt. Each population holds arbitrarily ten individual samples, which chose carefully to ensure homogenous distribution across the population. The studied wild taxa had recognised by the Botany Department,

Table 1. Accession number and GPS data of three *Calligonum* populations

Population	Location	Governorate	NGB accession number	Coordinates	
				latitude (N)	longitude (E)
MM	Mutubas	Kafr El-Sheik	C0021W	31°17'42"	30°31'8"
QQ	Qalabshu	El-Dakahlia	C0022W	31°30'51.21"	31°20'54"
GG	Gamasa	El-Dakahlia	C0023W	31°25'57"	31°31'08"

Explanations: MM = Mutubas population, QQ = Qalabshu population, GG = Gamasa population.
Source: own elaboration.

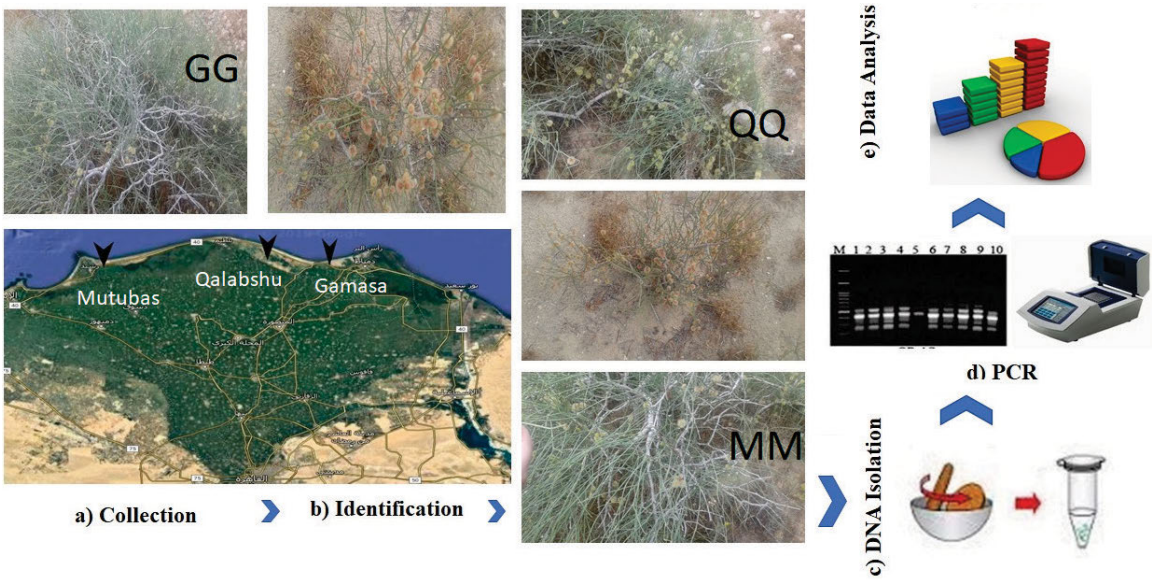


Fig. 1. Schematic diagram of the experimental procedures; GG, MM, QQ as in Tab. 1; source: own elaboration

Faculty of Science, Mansoura University, Egypt. The terminology of plant characters is according to Stearn [1973]. Fresh leaves were also arbitrarily collected to process the DNA extraction for molecular studies.

DNA EXTRACTION

Genomic DNA was extracted from young dried leaves of the thirty individuals of *C. polygonoides*. The samples were immersed in liquid nitrogen and crushed using a sterile mortar and pestle to get a fine powder [Dellaporta *et al.* 1983]. The quality of the extracted DNA was determined using gel electrophoresis and Nanodrop 8000 Spectrophotometer (Thermo Scientific, Wilmington, USA). Isolated genomic DNA was preserved at –80°C.

POLYMERASE CHAIN REACTION (PCR)

PCR amplification was used with five random 10-mer arbitrary primers synthesised by Operon Biotechnologies, Inc. (Germany) as shown in Table 2. The amplification has followed the procedure of Williams *et al.* [1990; 1993]. A total volume of 30 mm^3 of PCR reaction mixture follows 15 mm^3 of FideliTaq PCR Master Mix (USB Corporation, Cleveland, OH), a final concentration of 200 µM of each dNTPs and 1.5 mM $MgCl_2$, 1.0 µM of each primer (Eurofins MWG Operon, Germany), 2 mm^3 of genomic DNA, adjusted with sterile distilled water (up to 30 mm^3). The PCR amplification was performed with a Veriti 96 well plate thermal cycler (Applied Biosystems) as follows: 95°C for 1 min, followed by 35 cycles of 95°C for 30 s, 51°C for 30 s, and 68°C for 1 min, followed by a final elongation step. The PCR products had electrophoresed on 1.2% agarose gel [Sambrook *et al.* 1989]. Gels were photographed with a digital camera using a transilluminator, scanned, and analysed with Bio-Rad Video Gel documentation 2000.

Table 2. Primers names of random amplified polymorphic DNA (RAPD), their nucleotide sequences and size range

No.	Primer	Nucleotide sequence 5`to 3`	Size range (bp)
1	OP-A7	GAAACGGGTG	245–1200
2	OP-A9	GGGTAACGCC	245–1050
3	OP-B5	TGCGCCCTTC	340–940
4	OP-C4	CCGCATCTAC	145–1505
5	OP-D5	TGAGCGGACA	195–1385

Source: own elaboration.

DATA ANALYSIS

Profile of amplicons was scored as 1 if present or 0 if absent based on standard marker using Alpha Ease FCTM (version 4.0.1) software. The number of effective bands calculated according to the formula is $1/(p^2 + q^2)$; where p is the frequency of occurrence allele and q is the frequency of null allele. Shannon's information index was estimated as the formula: $I = -1[p \ln(p) + q \ln(q)]$. Heterozygosity ($h$) values for each dominant marker were calculated according to the formula by Anderson *et al.* [1993], $h = 1 - (p^2 - q^2)$; and unbiased diversity ($_uh$) was calculated by the formula: $_uh = [N/(N–1)]h$; where: p_i is the frequency of the i^{th} alleles, and q is the null allele frequency ($q = 1 - p$).

Genetic similarity was determined using the Jaccard coefficient [Jaccard 1908]. Algorithms of the un-weighted pair group method with arithmetic (UPGMA) averages are used to build trees [Nei, Li 1979] for studying the relationship among populations. A cophenetic matrix was derived from each matrix to test the goodness of fit of the clusters by comparing the matrices using the Mantel test [Mantel 1967]. The Mantel test with 1,000 permutations was estimated the association significance between the distance matrices resulting from molecular data of populations, AFLP, and combined analyses. Based on the averages of all attributes, the data set fed into SPSS (version 14.0), StatistiXL adding in Microsoft Excel (Kovach Computing Service 2013, version 1.8), GenAlEx (version 6.5) – Genetic Analysis in Excel] Program [Peakall, Smouse 2006; 2012].

RESULTS

GENETIC PARAMETERS REVEALED BY RANDOM AMPLIFIED POLYMORPHIC DNA (RAPD) ANALYSIS

Estimates of genetic parameters are vital management of entire *ex-* and *in-situ* conservation for the efficiency of a marker technique used in discriminatory populations based upon polymorphism that can detect. Some marker parameters had evaluated the informative and discriminative power of primer between thirty samples representing three populations of *Calligonum polygonoides* subsp. *comosum*, as shown in Figure 2. The RAPD analysis, as an arbitrarily discriminatory power, is implicated here to reveal data of genetic variabilities among three populations. A total of 129-amplicons oscillated from 145 to 1505 bp. A sum of 19-amplicons were specific markers with an average of nine bands for each population (Tab. 3). Despite no specific-RAPD amplicon appearance in population QQ, they showed the highest polymorphism (58%), except that MM population showed the lowest percentage of polymorphism (40%) with the highest number of specific markers (10 markers) scored. One present amplicon only is observed by primers OP-A7 and OP-B5 in genotype 6 with 665 bp and genotype 2 with 560 bp, respectively. Four positive amplicons were presented by primers OP-A9 (genotype 1 with 940 bp and 535 bp, genotype 9 with 615 bp, and genotype 2 with 590 bp) and OP-C4 (genotype 5 with 1505, genotype 7 with 1430 and genotype 2 with 1000 bp and 815 bp).

Some negative amplicons had observed by primers OP-C4 (genotype 10 scored 580 bp) and OP-A7 (genotype-5 scored 395, 295, and 245 bp). In the population of *GG*, one present specific marker in primers OP-A9 and OP-D5 was in genotype 1 with 245 bp and 1365 bp; three positive amplicons were in genotype 6 with 400 bp, genotype 8 with 840 bp, and genotype 9 with 1040 bp respectively, and four present unique amplicons were in genotype 2 with 590 bp, genotype 4 with 665 bp and 845 bp, and genotype 3 with 870 bp, respectively. In contrast, four negative amplicons were scored for genotype 2 with 310 bp in primer OP-A7, genotype 4 with 245 bp in primer OP-C4, and two negative bands in primer OP-D5, genotype 9 with 1055 bp, and genotype 3 with 625 bp.

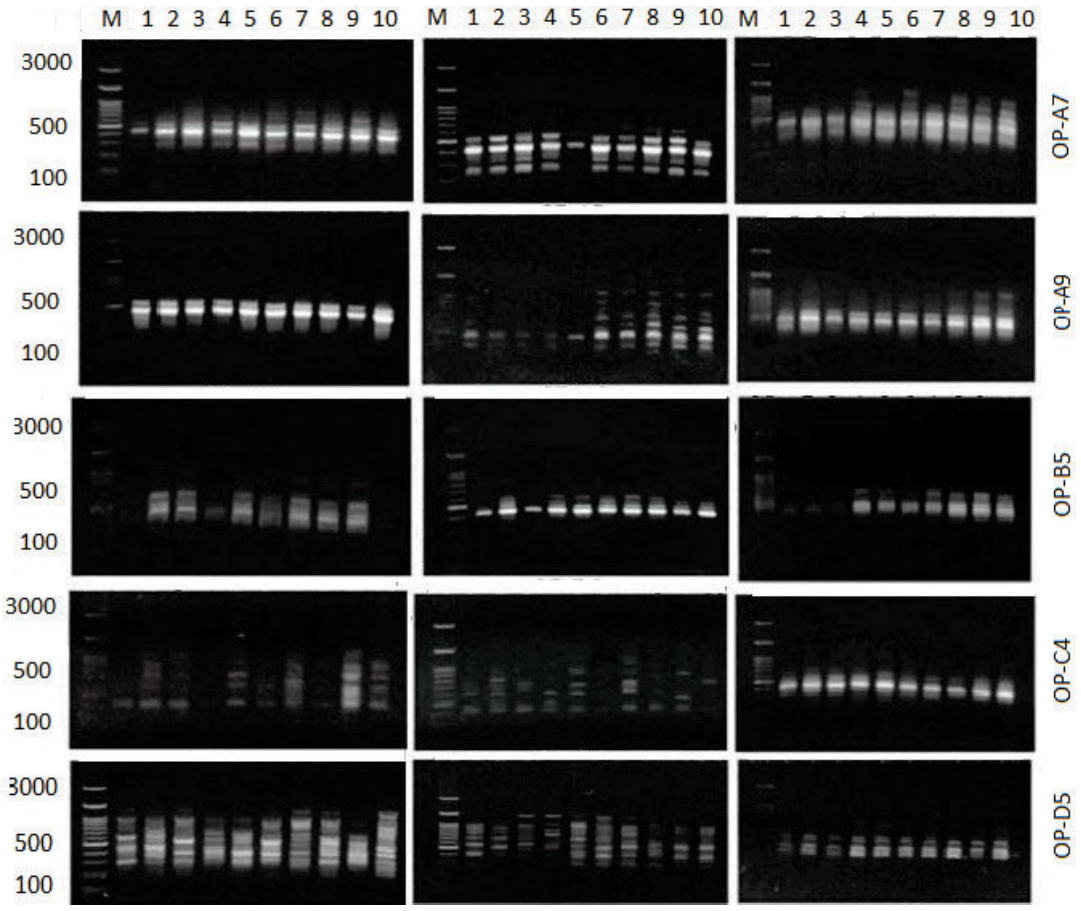


Fig. 2. The profiles of primers of random amplified polymorphic DNA (RAPD) used for screening individuals of *Calligonum polygonoides*; source: own study

Table 3. Allele frequency, specific bands of analysis of random amplified polymorphic and diversity of each population of *C. polygonoides* subsp. *comosum*

Population	Range (bp)	T_b	N_b	S_m	N_e	I	h	$_uh$	%P
QQ	145–1365	52	0.698	**OP-A7:** 4 (870/3; 845 & 665/4; 590/2); ^-U_b: 310/2 **OP-A9:** 1 (245/10) **OP-C4:** 3 (1040/9; 840/8; 400/6); ^-U_b: 245/4 **OP-D5:** 1 (1365/1); ^-U_b: 1055/9; 625/3	1.200	0.163	0.112	0.125	26.42
MM	245–1505	53	1.736	**OP-A7:** 1 (665/6); ^-U_b: (395 & 295 & 245/5) **OP-A9:** 4 (940 & 535/1; 615/9; 590/2) **OP-B5:** 1 (560/2) **OP-C4:** 4 (1505/5; 1430/7; 1000/2; 815/2); ^-U_b: 580/10	1.418	0.376	0.248	0.276	73.58
GG	580–1200	24	1.698	0	1.421	0.372	0.247	0.275	71.70
Mean	145–1505	8.9	1.38	1.267	1.346	0.304	0.203	0.225	57.23

Explanations: QQ, MM, GG as in Tab. 1, T_b = total band, S_m = specific marker, ^-U_b = negative unique band, N_e = No. of effective bands, I = Shannon's information index, h = diversity, $_uh$ = unbiased diversity.
Source: own study.

The average Shannon index (I) was 0.3 for the whole population, the lesser value was in population QQ (0.16), and the major was 0.38 for population MM. The N_e ranging from 1.2 for population QQ to 1.42 for the rest populations averaged 1.35 per population. The diversity (h) is of utmost important polymorphism information content (*PIC*) is given a high informative marker value and used to detect the level of heterozygosity in a population. The h values oscillating from 0.11 to 0.248 averaged 0.2. This variance is due to one or more reasons which might probably reflect the inbreeding and/or selection against hetero-

zygotes. The nature of markers used in the current study might also contribute to the observed level of heterozygosity resulting in the non-detection of homozygotes from heterozygotes due to the presence of null alleles.

GENETIC SIMILARITY AND CLUSTER ANALYSIS

The similarity matrices based on Jaccard coefficient, as given in Table 4, were calculated for each population and all populations. The similarity matrices directly recorded positive values except for population QQ ranging from –0.133 to identical similarity (1.000). The population *GG* scored the highest similarity values between individuals 2 and 5 (0.786), followed by between individuals 8 and 6 (0.759), and between accession 8 and 7 (0.663). On the other hand, it scored the similarity (0.150) between individuals 9 and 3, and individuals 9 and 2. The population *MM* has recorded the highest similarity values between individuals 1 and 3, individuals 3 and 4, and individuals 8 and 9 (0.774, 0.740, and 0.717, respectively). It scored the lowest values between individuals 2 and 8 (0.169).

The population *QQ* has scored the highest values between individuals 9 and 10, individuals 1 and 2, individuals 2 and 3, and individuals 6 and 7 (1.00, 0.917, 0.917, and 0.914, respectively). It recorded the lowest similarity between individuals 5 and 9, 5 and 10, 4 and 9, 4 and 10, and 3 and 9, 3 and 10 (–0.133, –0.019, and –0.020, respectively). The generated similarity by the RAPD data set of ARTA populations had evaluated the genetic distance between individuals revealing high similarity. The highest

Table 4. Similarity values between individuals of each population

Individuals	Individuals								
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
Gamasa population									
2	0.459								
3	0.296	0.660							
4	0.527	0.378	0.296						
5	0.480	**0.786**	0.624	0.559					
6	0.467	0.307	0.390	0.467	0.417				
7	0.348	0.441	0.527	0.431	0.588	0.596			
8	0.407	0.287	0.455	0.326	0.419	**0.759**	**0.663**		
9	0.214	**0.150**	**0.150**	0.296	0.380	0.390	0.527	0.371	
10	0.402	0.303	0.391	0.402	0.469	0.555	0.512	0.532	0.391
Mutubas population									
2	0.643								
3	**0.774**	0.583							
4	0.509	0.597	**0.740**						
5	0.398	0.399	0.555	0.585					
6	0.312	0.249	0.416	0.497	0.368				
7	0.342	0.236	0.550	0.449	0.389	0.578			
8	0.233	**0.169**	0.258	0.340	0.211	0.672	0.666		
9	0.346	0.292	0.367	0.375	0.248	0.392	0.548	**0.717**	
10	0.480	0.388	0.491	0.362	0.320	0.506	0.606	0.664	0.699
Qalabshu population									
2	**0.917**								
3	0.826	**0.917**							
4	0.516	0.580	0.699						
5	0.589	0.659	0.768	0.724					
6	0.411	0.302	0.411	0.724	0.635				
7	0.489	0.389	0.489	0.649	0.734	**0.914**			
8	0.294	0.128	0.083	0.058	0.009	0.225	0.388		
9	0.210	0.020	**–0.020**	**–0.094**	**–0.133**	0.102	0.292	0.871	
10	0.210	0.020	**–0.020**	**–0.094**	**–0.133**	0.102	0.292	0.871	**1.000**

cont. Tab. 4

Individuals	Individuals								
	1	2	3	4	5	6	7	8	9
All populations									
2	**0.613**								
3	0.572	**0.675**							
4	0.516	0.482	0.518						
5	0.464	0.592	0.608	0.597					
6	0.394	0.278	0.388	0.518	0.433				
7	0.369	0.349	0.511	0.455	0.522	**0.648**			
8	0.315	**0.198**	0.293	0.303	0.271	**0.631**	0.595		
9	0.268	**0.179**	0.210	0.289	0.255	0.340	0.468	0.595	
10	0.394	0.285	0.375	0.318	0.321	0.445	0.475	0.634	**0.615**

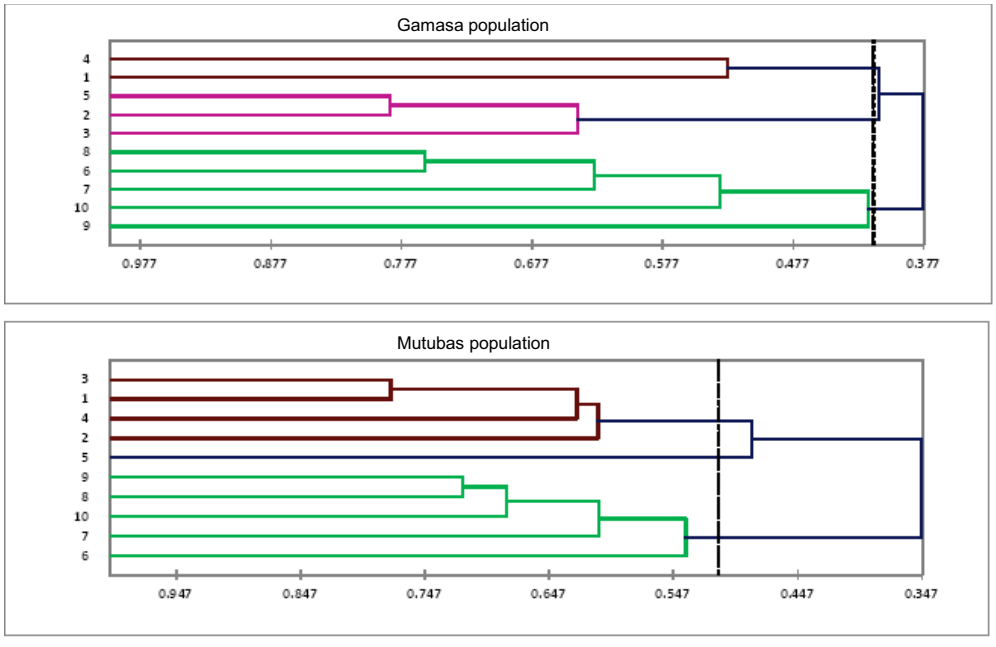
Source: own study.

similarity scored between individuals 2 and 3, 6 and 7, 6 and 8, 9 and 10, and 1 and 2 (0.675, 0.648, 0.631, 0.615, and 0.613, respectively). In contrast, the lowest values scored between individuals 2 and 9, and 2 and 8 (0.179 and 0.198, respectively).

A phenogram generated by UPGMA cluster analysis based on Nei and Li coefficient [Nei, Li 1979] showed a grouping of ten individuals of *C. polygonoides* subsp. *comosum* populations (Fig. 3) for each Arta population and together. The population GG split into two groups at a distance of 0.377. Each group split further into two subgroups which split further into sub-sub groups. Ten objects could be divided into three classes, as shown in Table 5. Five objects of 9, 10, 7, 6, and 8 clustered into one group at a distance of 0.4197. Three objects of 3, 2, and 5 had grouped into one group at a distance of 0.642. The last group included a distance of 0.527 between two objects of 1 and 4.

Investigations of population *M* split into two groups at a distance of 0.348. Objects of population *MM* had classified into three class-centroid classes, as given in Table 5. The first one isolated 6, 7, 10, 8, and 9 at a distance of 0.537. Object 5 only filled in a subgroup at a distance of 0.484. The last class has consisted of 2, 4, 1, and 3 at a distance of 0.61. Investigations of population QQ had wide-highly put at a distance between objects. They are divided into two groups at a distance of 0.092 and three classes. Four objects that are 6, 7, 4, and 5 had isolated at a distance of 0.685 in a class. Three objects that are 3, 1, and 2 had separated at a distance of 0.871 in the second class. The third class isolated the objects of 8, 9, and 10 at a distance of 0.87. The highest similarity has recorded between 9 and 10 at a distance of 1.00.

The investigations in all derived data had divided into two main groups in three classes of objects at a distance of 0.34. The first class includes objects 4, 1, 5, and 2 at a distance of 0.528. The second one consists of two objects 6 and 7, at a distance of 0.648. The last class includes objects of 9, 8, and 10 at a distance of 0.605. The distances between these classes had given in Table 5 according to the central objects.



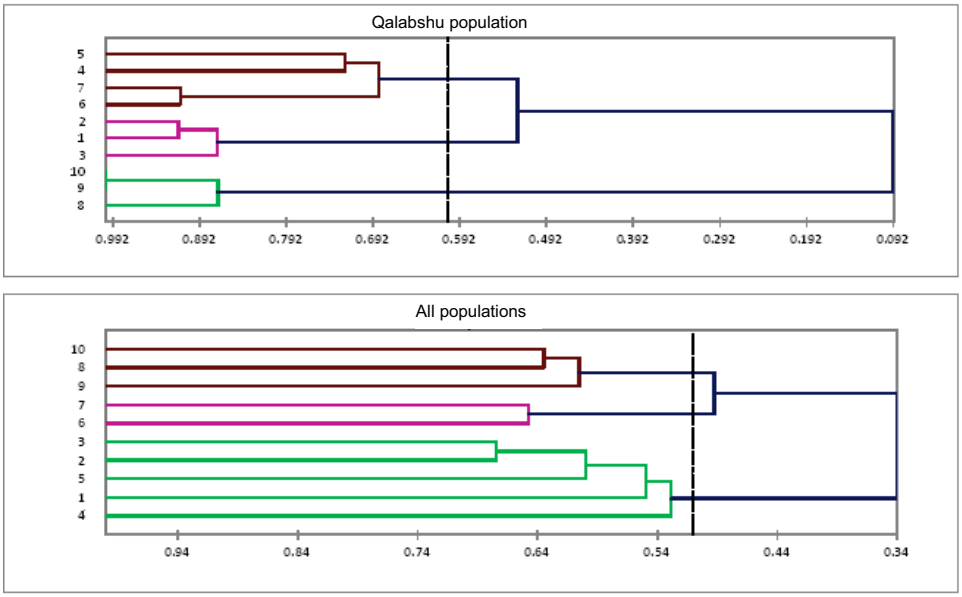


Fig. 3. Phenogram generated by un-weighted pair group method with arithmetic (UPGMA) for ten individuals representing three populations; source: own study

Table 5. Distances between the central objects regenerated by un-weighted pair group method with arithmetic cluster analysis

Class	Class		
	1	2	3
Mutubas population			
1	0.000		
2	3.464	0.000	
3	4.583	4.583	0.000
Qalabshu population			
1	0.000		
2	2.828	0.000	
3	3.317	3.000	0.000
Gamasa population			
1	0.000		
2	4.000	0.000	
3	4.359	3.606	0.000
All populations			
1	0.000		
2	6.245	0.000	
3	6.325	5.745	0.000

Source: own study.

Table 6. Mantel test for all combinations of the genetic distance matrices

Population	*r*	*p*-value[1)]
GG vs MM	0.3420	2.7%
GG vs QQ	0.3829	1.7%
GG vs All	0.7144	0.01%
MM vs QQ	0.6347	0.01%
MM vs All	0.8340	0.01%
QQ vs All	0.8340	0.01%

[1)] Significance level is at 5%.
Explanations: QQ, MM, GG as in Tab. 1, *r* = correlation coefficient.
Source: own study.

The Mantel test with 1,000 permutations had estimated for determining the level of correlation (Tab. 6). We also found a high correlation (r = 0.83) between the population *QQ* and population *MM* matrices followed by the population *GG* matrix than between the population *GG* and population *MM* matrices (r = 0.34), with both associations being significant at p = 0.05. Such difference in correlation among matrices could be independent of the different number of data points for RAPD markers and collected region characteristics. Our results suggested that to obtain an extra complete understanding of the degree of each genotype divergence in each population affected by its region. It is necessary to consider the molecular data, not separate combining into the collected locality. Preferable individual discrimination of the Arta population is obtained by the combined molecular profile and region data when one determines, a priori, the minimum number of markers that will lead to the same results as the combination of all characteristics.

DISCUSSION

GENETIC PARAMETERS OF *Calligonum* POPULATIONS

Genetic parameters give more informative and discriminative power of a marker between *Calligonum* populations managing entire *ex-* and *in-situ* conservation. The efficiency of a marker technique used in discriminatory *Calligonum* populations has based on polymorphism that can detect.

The heterozygosity (*h*) represents the direct count of heterozygosity in the population and is estimated based on the allele frequency of individuals given that population according to Hardy–Weinberg equilibrium. The *PIC* has been used usually for evaluating the informative potential of markers in different accessions and germplasm [Grativol *et al.* 2011; Gougerdchi *et al.* 2014; Mahdy 2018; Naceur *et al.* 2012; Xu *et al.* 2017; Yadav *et al.* 2015]. Botstein *et al.* [1980] had divided the value of heterozygosity *h* into three classes. The highly informative markers of a locus have values >0.50 with many alleles is the desirable marker for genetic diversity. The reasonably informative marker of a locus has values between 0.25–0.50 in a moderately informative locus. The slightly informative marker of a locus that has values <0.25 is the lowest informative locus. The Shannon information index (*I*) is one of the foremost genetic diversity measurements [Sherwin *et al.* 2006; Varshney *et al.* 2007]. The effective number of bands (N_e) is a reciprocal of gene homozygosity [Hartl, Clark 1997]. The N_e is used to corollary the *h*; when the *h* is high, N_e will be higher [Varshney *et al.* 2008].

Molecular markers proved a valuable characterisation tool of the genetic diversity in a species. Various molecular markers might reveal different classes of polymorphism [Kim *et al.* 2019; Powell *et al.* 1996; Russell *et al.* 1997; Sun, Zhang 2012; Xiang *et al.* 2015]. Various molecular markers had utilised for detecting the *Calligonum* genetic diversity [Dhief *et al.* 2011; Ren, Tao 2002; Soskov 2011; Song *et al.* 2020; Tao, Ren 2004; Vyas *et al.* 2012]. Our results agreed with Ren and Tao [2002], Dhief *et al.* [2011], and Soskov [2011]. Vyas *et al.* [2012] reported that 90% of RAPD-marker was polymorphic. The high level of genetic diversity between various *Calligonum polygonoides* collections.

GENETIC SIMILARITY AND CLUSTER ANALYSIS

The variation rate that discriminates between various populations, as revealed by the RAPD marker, maybe due to the diversity in the source of the detected diversity. RAPD markers target the different loci in the genome [Tomka *et al.* 2013; Wang *et al.* 2010]. The molecular characterisation and evaluation help in the identification of the variance of populations that can utilise in expressing the desired divergence. Morphological characters may affect environmental conditions [Abdel Khalil *et al.* 2014]. The use of molecular markers has become a common practice in studies of population structure, genetic diversity for breeding, and improvement programs, especially when if it is desirable to transfer new genes into cultivated crops [Langridge, Chalmers 2004] and in selecting one or more appropriate way to conserve the species *in-* or *ex-situ*. In addition, these markers are most useful in different disciplines such as genetic diversity, phylogenetic studies, gene tagging, genome mapping, and evolutionary biology in a wide range of plant species. Vyas *et al.* [2012] reported the RAPD patterns had not systematic been in the classification of sampling with the same regions leading to an uncharacterised diversity in the dendrogram.

Our results showed that these gene pools are a valuable reservoir for enriching genetic biodiversity due to the adaptability of *Calligonum* species. The information on a species structure and parameter can provide a piece of basal information for the conservation of the biodiversity of species. These structures and parameters are vital tools in assessing the sustainable conservation and utilisation of a species and the management of ecosystems. Long-term conservations rely on the availability and knowledge of the genetic parameters of a species. Also, the richness of a species in a target region is the most commonly used measure of biological diversity and can delineate protected areas, monitor biological systems, and investigate environmental relationships. A survey for a target region plays a vital role in determining numerous estimators that propose to collect and conserve the negative and positive bias of underdone main objects. Therefore, biodiversity in a population regulates the process of survey and collection for the long-term conservation of a dominant and polydomain species in a target area. Biodiversity helps determine the relationship between all species and best predict the possibility of evolutionary time from past to future in the area.

CONCLUSIONS

The *Calligonum* species that habituate in banks of water sources, wastelands, roadsides, and agricultural fields are weed out by humans ignorant of their economic significance or due to the lack of a local market. Dominant species, including *Calligonum* species, could stabilise the shifting dunes via consisting of a dense network of roots. Dominant species in a region can be utilised in afforestation in the same region and/or other places which have the same environmental conditions. Fragmentation of habitats, excessive grazing, invasive species, and climate change destroy *Calligonum* genetic diversity. Investigations on endangered species indicated alarming genetic erosion levels and deteriorating population bulks below minimum viable limits. Recovery and conservation programs begun by authorised administrations have reduced genetic erosion. Cultivation helped relieve harvest pressure on wild flora and preserve the genetic diversity of some species. The presence of numerous species, lack of adequate research funds, loss/degradation of natural populations, and utilisation of genetic resources with benefit-sharing are the concerns that need to resolve for conserving *Calligonum* genetic diversity. The biodiversity in the species population is regulated and can affect long-term factors such as community stability and evolutionary time, as heterogeneity of both micro-climate and macro-climate affects the diversification among the different communities. Collection of species diversity is required to do what actions for assuring to identify and quantify for long-term conservation and sustainable utilisation. The dominant species in a region can be utilised in afforestation in the same region and/or other places which have the same environmental conditions.

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