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Full Length Research Paper

Natural genetic variation in *Calligonum* Tunisian genus analyzed by RAPD markers

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The *Calligonum* genus is one of the most economically important resources of the Tunisian desert, playing an important role in the lives of desert local population. A great range of genetic diversity could be seen in diverse populations of this genus which are spread all over Tunisian areas. DNA-based molecular markers are playing increasingly important role in the analysis of genetic diversity in wide range of plants. This study is an attempt to collect, compile and collate information on the existing natural genetic diversity, at intra-specific and inter-populational levels in *Calligonum* genus (*C.azel*, *C.arich* and *C.comosum*). This study deals with 16 genotypes of *Calligonum* (Polygonaceae) collected from different regions of Tunisian desert using random amplified polymorphic DNA (RAPD) marker. In total, 46 polymorphic bands amplified by 4 random primers, with the polymorphic rate of 89.06% were recorded. The commercial software package SPSS 16 was used to develop similar matrices based on the Dice coefficient which is defined as 2a/2a+u, where "a" is the number of positive matches and "u" is the number of non-matches. From the analysis, it is imperative that predominantly obligate out breeding behavior of *Calligonum* genus, helps these diverse accessions to spread and occupy specific geographical niches in the Tunisian arid regions.

Key words: *Calligonum*, genetic diversity, Inter-specific variation, RAPD marker, Tunisian desert.

INTRODUCTION

Desertification is a land degradation problem of major importance in the world's arid regions. Approximately, three quarters of Tunisia are arid and desert regions (Le Houérou, 1959). Virtually, all of the rangeland has suffered severe land degradation. *Calligonum* genus, bestows the status of key-stone species of Tunisian desert, an important source of animal food for sustenance during frequently occurring famines and is also valued for commercial and medicinal purposes (Bhandari, 1995). *Calligonum* genus with smooth stems and branches, bearing abortive flowers and small succulent fruits, is eaten when food is scarce during famines in arid regions of Tunisia. Flowers made into bread or cooked with clarified butter or coconut oil to make a local delicacy (Bewal and Sharma, 2008). Roots and thick branching stems are used as fuel (Singh and Wadhwani, 1996). The aqueous paste of plant acts as an antidote against the heavy doses of opium and also against poisonous effects of certain harmful plants (Singh and Wadhwani, 1996). *Calligonum* species (*C.azel*, *C.arich* and *C.comosum*) forms communities with other dominating species at the top of sand dunes and stabilizes the shifting sand dunes, as it consists of dense network of roots. One of the basic requirements for the sustainable and optimal use of the available meager resources of Tunisian desert region is to characterize and estimate the existing genetic diversity in natural populations. However, *Calligonum* genus did not receive the due attention it deserves as a key stone species of Tunisian desert. From cytological point of view, there is a single report by Valovich et al. (1973), that proposed 2n = 54 as somatic chromosome number. No chromosome number reports for *Calligonum* genus or any other species of the genus *Calligonum* from Tunisia are available as yet. Examination of morphological characteristics is the standard method of identifying desert

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Table 1. *Calligonum* accessions studied with their origin.

Accession	Code	Site	Altitude (m)	Origin	Co-ordinate
C.azel	P7	Jbil	169	Kebeli	331410.20 N / 92505.08 E
C.comosum	P14	Oued Zar	334	Tataouine	212935.35 N / 0100045.97 E
C.azel	P2	El Borma	242	Tataouine	320330.06 N / 0 90726.98 E
C.azel	P4	Tiert	392	Tataouine	304642.35 N / 0101115.45 E
C.azel (cultivited)	P11	El Fjé	15	Medenine	332959.05 N / 0103829.99 E
C.comosum	P8	Oued Jnein	280	Tataouine	314822.65 N / 0101615.92 E
C.comosum	P10	Lybia	484	Lybia	314439.38 N / 0110147.68 E
C.comosum	P13	El Borma	242	Tataouine	320330.06 N / 090726.98 E
C.comosum	P3	Tiert	392	Tataouine	304642.35 N / 0101115.45 E
C.comosum	P6	Oued Dhabaa	249	Kebeli	325314.79.E / 3677679.32 N
C.comosum	P15	Ksar Ghilane	240	Kebeli	325936.06 N / 093901.84 E
C.comosum	P16	Oued Zridib	369	Medenine	331516.01 N / 095849.11 E
C.comosum	P9	Jbil	169	Kebeli	331410.20 N / 92505.08 E
C.comosum (cultivited)	P12	El Fjé	15	Medenine	332959.05 N / 0103829.99 E
C.comosum	P5	El Ouera	188	Tataouine	325629.28 N / 0102749.46 E
C.arich	P1	El Borma	242	Tataouine	320330.06 N / 0 90726.98 E

species, but not all of them can be distinguished on this basis. Several techniques have been used to complement morphological examination of desert plants, and most of them rely on variations among isoenzymes (Laugesen et al., 2007) and seed storage proteins (Cansi et al., 2003). Nevertheless, characterization with these kinds of markers is not very efficient for barley varieties due to the low levels of allelic variation in many isoenzymatic loci, and to the high degree of genetic relationship among the different varieties, and also to the high degree of polymorphism within desert species.

Molecular markers have been proved to be valuable tools in the characterization and evaluation of genetic diversity within and between species and populations. It has been shown that different markers might reveal different classes of variation (Powell et al., 1996; Russell et al., 1997). It is correlated with the genome fraction surveyed by each kind of marker, their distribution throughout the genome and the extent of the DNA target which is analyzed by each specific assay (Dávila et al., 1999). The advent of the polymerase chain reaction (PCR) favors the development of different molecular techniques such as random amplified of polymorphic DNA (RAPD), simple sequence repeats (SSR or microsatellite), sequence tagged sites (STS), random amplified microsatellite polymorphism (RAMP) and inter-simple sequence repeat polymorphic DNA (ISSR), and so on (Wu et al., 2004; Guasmi, 2010). These molecular markers have been used in *Calligonum* genus for detecting genetic diversity, genotype identification, genetic mapping (Fernández et al., 2002). Of these techniques, RAPD has several advantages, such as simplicity of use, low cost, and the use of small amount of plant material, etc. RAPDs proved to be useful as genetic markers in the case of self-pollinating species with a relatively low level of intraspecific polymorphism, such as hexaploid wheat (Joshi and Nguyen, 1993). Investigations on relationship and clustering of *Calligonum* species through isozyme analysis have been reported lately by Tao and Ren (2004). RAPD analysis to assess inter-specific relationship in *Calligonum* genus collections from Tunisia has been attempted by Ren and Tao, (2002). Here, RAPD is used to assess the genetic diversity and determine the genetic relatedness / distance among various accessions of Tunisian *Calligonum* genus.

MATERIALS AND METHODS

Plant material

16 *Calligonum* accessions selected from the natural Tunisian desert were used in this study. The accessions number and sites are listed in Table 1 and shown in Figures 1a and b.

DNA isolation

Total DNA was isolated from fresh leaves as described by Doyle and Doyle (1990) with some modification. DNA concentration was determined by both spectrophotometry at 260 nm and by 2% agarose gel electrophoresis.

RAPD-PCR analysis

RAPD analysis was carried out with 10 decamer random primers from Operon molecular for life (Table 2). PCR amplifications were carried out also with 3 accessions. The primers that give clear and polymorphic amplification patterns (4 primers) were used for further analysis with all the 16 accessions. For each primer, a 20 µl amplification reaction contained: 100 ng of genomic DNA, 5 mM of $MgCl_2$, 1U of Taq DNA polymerase and 4 µl of buffer (Taq Buffer

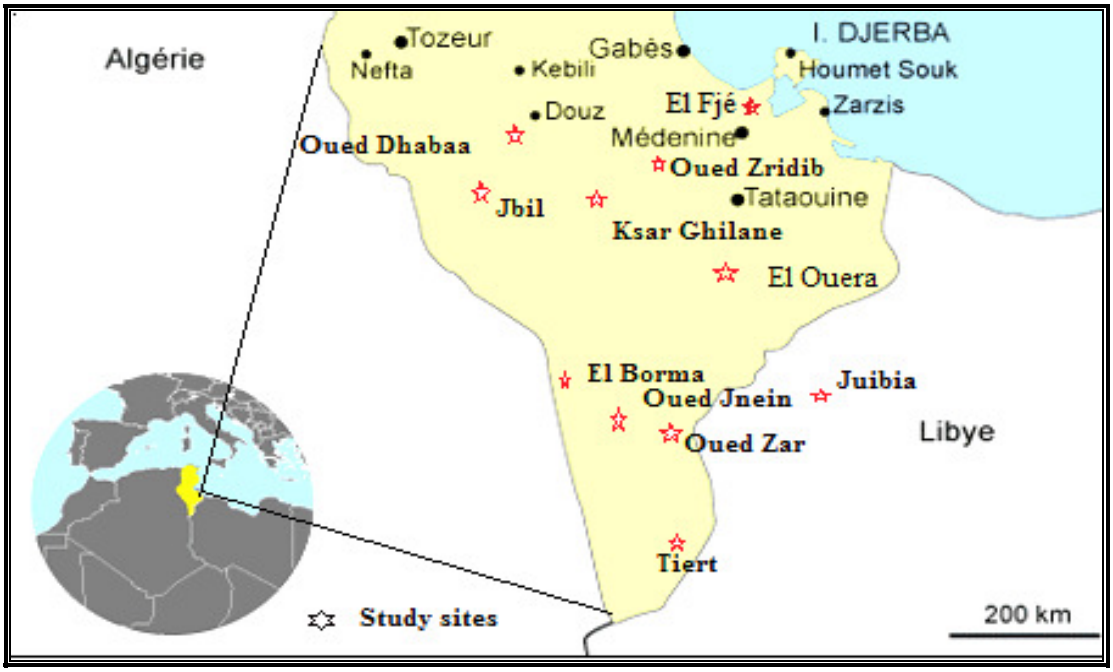

Figure1a. Collecting sites of *Calligonum* accessions.

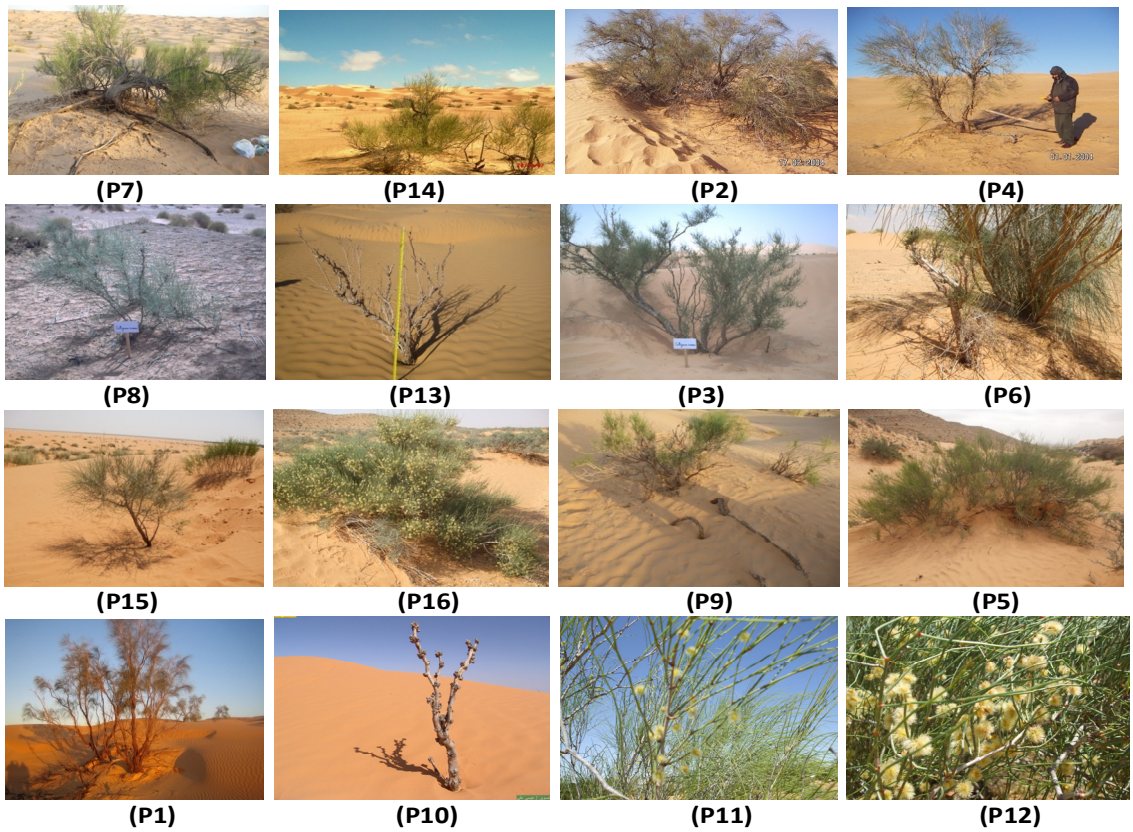

Figure1b. Natural sites of *Calligonum* accessions: **(P7)***C.azel* Jbil, **(P14)** *C.azel Kamour*, **(P2)***C.azel* ElBorma, **(P4)***C.azel* Tiert, **(P8)** *C.comosum* Oued Jnein, **(P5)** *C.comosum* ElBorma, **(P3)** *C.comosum* Tiert, **(P6)** *C.comosum* Oued Dhabaa, **(P15)** *C.comosum* Ksar Ghilane, **(P16)** *C.comosum* Oued Zridib, **(P9)** *C.comosum* Jbil **(P13)** *C.comosum* ElOuera **(P1)** *C.arich* ElBorma **(P10)** *C.comosum* Lybia, **(P11)** *C.azel* El Fjé, **(P12)** *C.comosum* El Fjé.

Table 2. RAPD primers tested in this study.

S/N	Primer	Primer sequence (5'-3')
1	UBC-402	CCCGCCGTTG
2	UBC-475	CCAGCGTATT
3	UBC-490	AGTCGACCTT
4	UBC-534	CACCCCCTGC
5	AT	CAGTGGTTCC
6	W07	CTGGACGTCA
7	OBP12 (A1)	CCTTGACGCA
8	OPY15 (A9)	AGTCGCCCTT
9	AF14	GGTGCGCACT
10	AX16	GTCTGTCGG

with $(NH_4)_2SO_4$ 10×). PCR amplifications were performed in a gen-Amp PCR 9700 thermal cycler system. The PCR conditions include initial denaturation at 94°C for 5 min, followed by 45 cycles: denaturation at 92°C for 1 min, annealing at 50 °C for 2 min, extension at 72°C for 2 min with final extension at 72°C for 7 min.

Reproducibility of amplification patterns

DNA amplifications with each RAPD primer are repeated at least three times to ensure reproducibility. The bands were considered reproducible and scorable only after observing and comparing them in three separate amplifications for each primer. Clear and intense bands were scored while faint bands against background smear were not considered for the further analysis.

Scoring and data analysis

For each accession, each fragment / band that was amplified using RAPD primer is treated as a unit character. Unequivocally scorable and consistently reproducible amplified DNA fragments are transformed into binary character matrices (1 for presence, 0 for absence). The commercial software package SPSS 16 is used to develop similar matrices based on the Dice coefficient which is defined as 2a/2a+u, where "a" is the number of positive matches and "u" is the number of non-matches. These data are then used to construct dendrogram for cluster analysis. One dendrogram for RAPD data is generated. Molecular weight of each of the potential specific bands is calculated using the software program Gel pro analyser. The distance matrices obtained in RAPD analysis are compared using correlation analysis. Band informativeness (Ib) and resolving power (Rp) are calculated as given by Prevost and Wilkinson (1999). The formulae used for the aforementioned parameters are:

1. Band informativeness of a given band: $Ib = 1 - (2 \times |0.5 - p|)$

Where, p is the proportion of the total genotypes containing the band.

2. Resolving power of a primer: $Rp = \sum Ib$

Analysis in main components (ACP)

The ACP is a technique commonly used to visualize data and finding the true measurements of other data's sampling. The ACP generates entirely new variables (axes) and new linear combinations of the original variables, so that the maximal value of the restrained variance in the sampling (information) is concentrated in the first main components. This multivariate analysis is principally used to condense the initial variables and at least show the correlation between them in a set of independent synthetic variables that are linearly measured by the variable combinations. This analysis has been achieved by the software XLSTAT.02.2009.

RESULTS

Identification and evaluation of RAPD markers for diversity estimates in *Calligonum* genotypes

A total of 10 primers consisting of di and tri repeat motifs were used for the initial screening with 3 *Calligonum* genotypes. Out of these, 6 primers gave no amplification at all, while only 4 primers are found to give clear and polymorphic patterns, and are subsequently used to analyze the entire set of 16 genotypes. These primers are then used for RAPD analysis of all the 16 *Calligonum* genotypes. Amplification products of the 16 genotypes with these 4 primers yielded a total of 46 scorable bands, which are all polymorphic (Table 3 and Figures 2, 3, 4 and 5). The highest number of bands (16) is obtained with primer AX16, while the lowest number (8) is obtained with A9 and AF14 primers. A1 and A9 primers show the same variation in their ability to detect polymorphism (100%). The 4 polymorphic primers exhibit variation with regard to average band informativeness (AvIb) and resolving power (Rp). The AvIb and Rp values of these polymorphic primers have been depicted in Table 3. The primer AX16 showed the lowest AvIb (0.36) and the highest Rp (5.84) while the highest AvIb (0.45) and the lowest Rp (3.62) values are exhibited by the primers AF14

Genetic diversity and clustering pattern of 16 *Calligonum* genotypes, based on RAPD data

Three concentrations of DNA genomic (20, 40 and 100 ng) accessions of the 16 *Calligonum* kind of the Tunisian South have been tested with different uncertain primer sequences (oligonucleotides). Of this fact, 100 ng have been found as the best concentration for every PCR (Amplification reaction). Out of the different uncertain tested primers, four that were reproducible are A1, A9, AF14 and AX16. Primers of set A1 and A9 give several strips for the majority of samples of DNA while primers of set AF14 and AF16 give monomorphic bands mainly for the majority of DNA samples (Table 3).

Frequency of the RAPD strips

The obtained photos of gels are represented in Figures 2, 3, 4 and 5. The analysis of these profiles permits Table 3 to be raised. The observation of Figures 2, 3, 4 and 5

Table 3. Polymorphism exhibited by RAPD primers in *Calligonum* genus.

Primer	Temperature (°c)		RAPD-PCR band		Resolving power (Rp)	Average of informativeness band (Avlb)
	Theoretical	Optimal	Total	Polymorphic (%)		
A1	34	34	12	12 (100%)	4.93	0.41
A9	34	34	10	10 (100%)	4.1	0.41
AF14	34	34	8	6 (75%)	3.62	0.45
AX16	34	34	16	13 (81.25%)	5.84	0.36
Total			46	41 (89.06%)	M=18.49	M=1.63

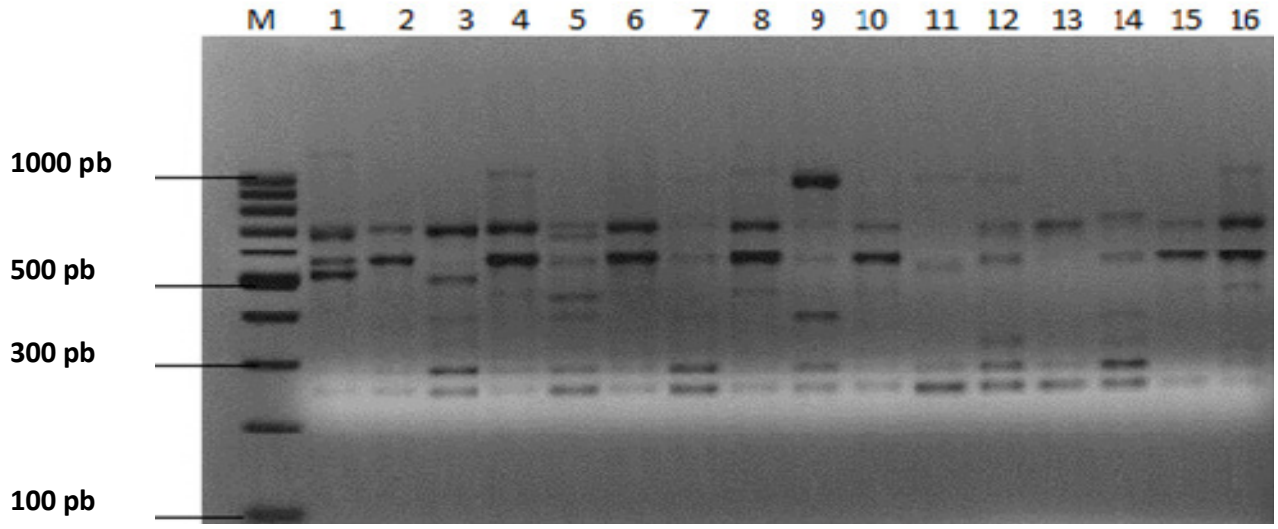

Figure 2. DNA Fragments amplified by RAPD while using the primer A1.

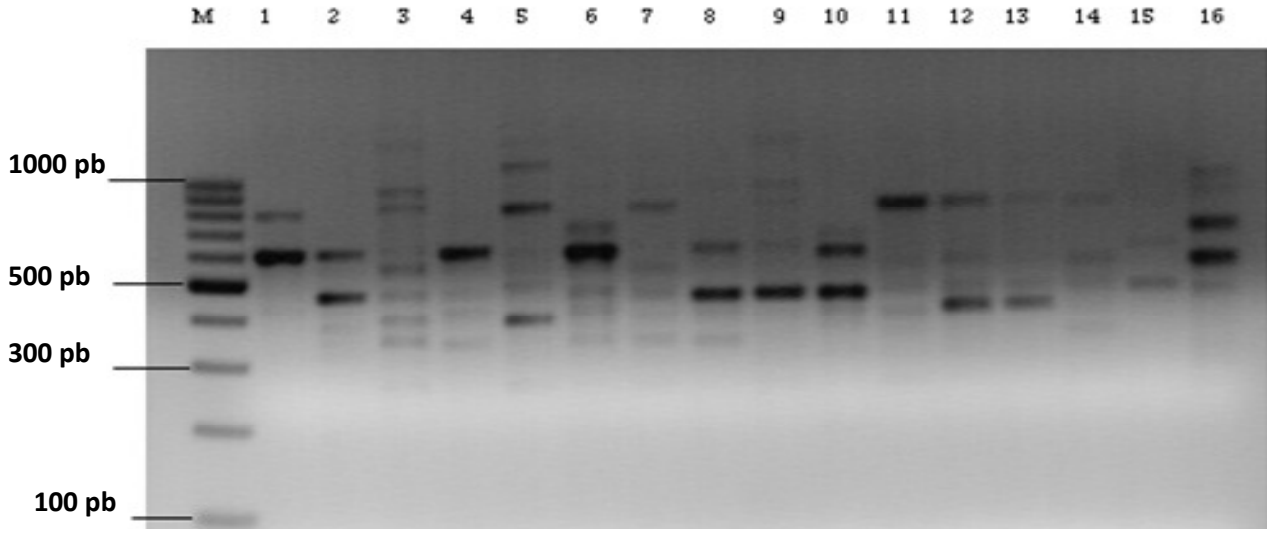

Figure 3. DNA Fragments amplified by RAPD while using the primer A9.

frosts showed that the four primers permitted the generation of the entire 46 bands with an average of 12 bands by primers. The size of bands of these four primers varies between 170 and 1300 pb. The primer A1 permits to generate 12 bands of sizes that vary between 270 and 1100 pb, whereas the primer A9 permits the

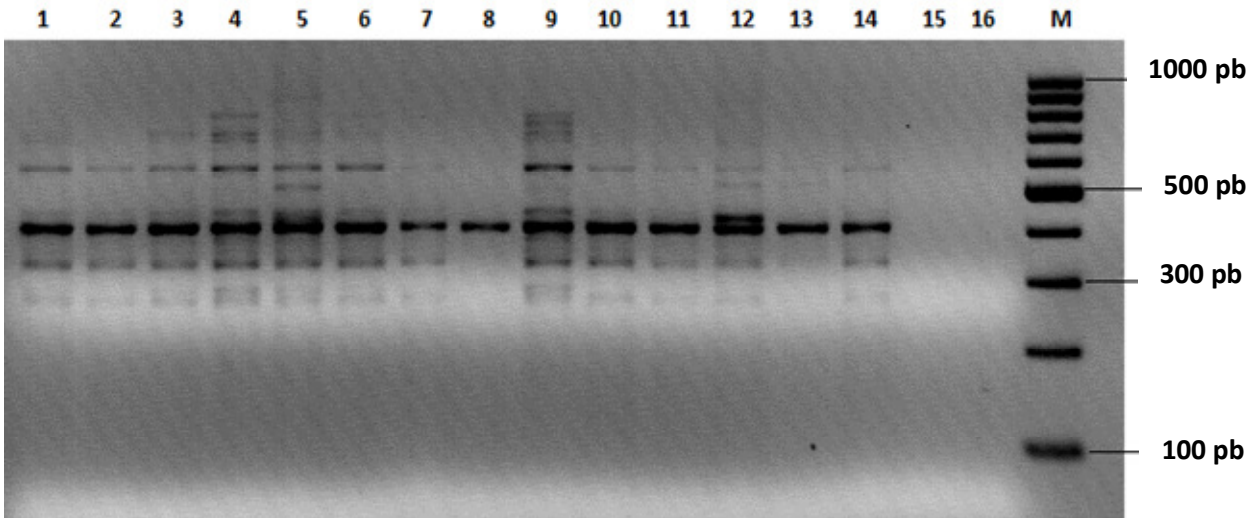

Figure 4. DNA fragments amplified by RAPD while using the primer AF14.

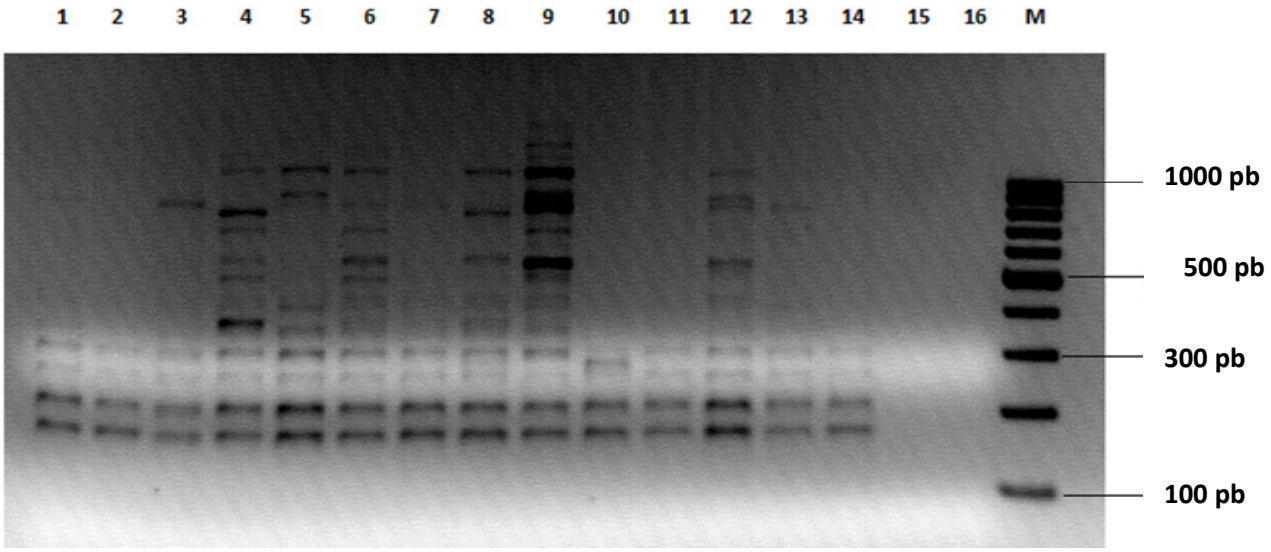

Figure 5. DNA Fragments amplified by RAPD while using the primer AX16.

amplification of 8 bands of sizes that vary between 350 and 1100 pb. The primer AF14 generated 8 bands of sizes that vary between 300 and 800 pb, whereas the primer AX16 generates 16 bands of sizes that vary between (170 and 1300 pb). From this picture, one can say that the four primers present a very elevated rate of polymorphism that can reach 100% for primers A1 and A9, whereas it can reach only 75 and 81.25% respectively for primers AF14 and AX16. Indeed, one notices that bands of sizes 270 and 700 pbs (primer A1), 450 and 600 pb (primer A9), 300, 350, 400 and 600 pb (primer AF14) and 170, 200, 250 and 300 pb (primer AX16) are the least polymorphous whereas bands of sizes 360 and 800 pb, (primer A1),dth 700 and 1100 pb (primer A9), of 500 and 800 pb (primer AF14) and 870, 1000, 1200 and 1300 pb (primer AX16) are considered the most polymorphous view that can distinguish individuals or an accession among others. For bands of size 300, 600 and 700 pb, one can say that they are the most frequent whereas bands of sizes 900, 1200 and 1300 bp are considered the least frequent. The observation of values of informative band average and the resolving power (RP) confirmed the fact that the used bootjacks show an important variation. The primer AF14 possesses the value of the most elevated AVIb with 0.45 whereas the primer AX16 possesses the weakest value with 0.36. So for the RP, one notes that the present primer AX16 is the most elevated value 5.84 whereas the present primer AF14 is the least elevated value with 3.62 and in short primers A1 and A9 present some intermediate values

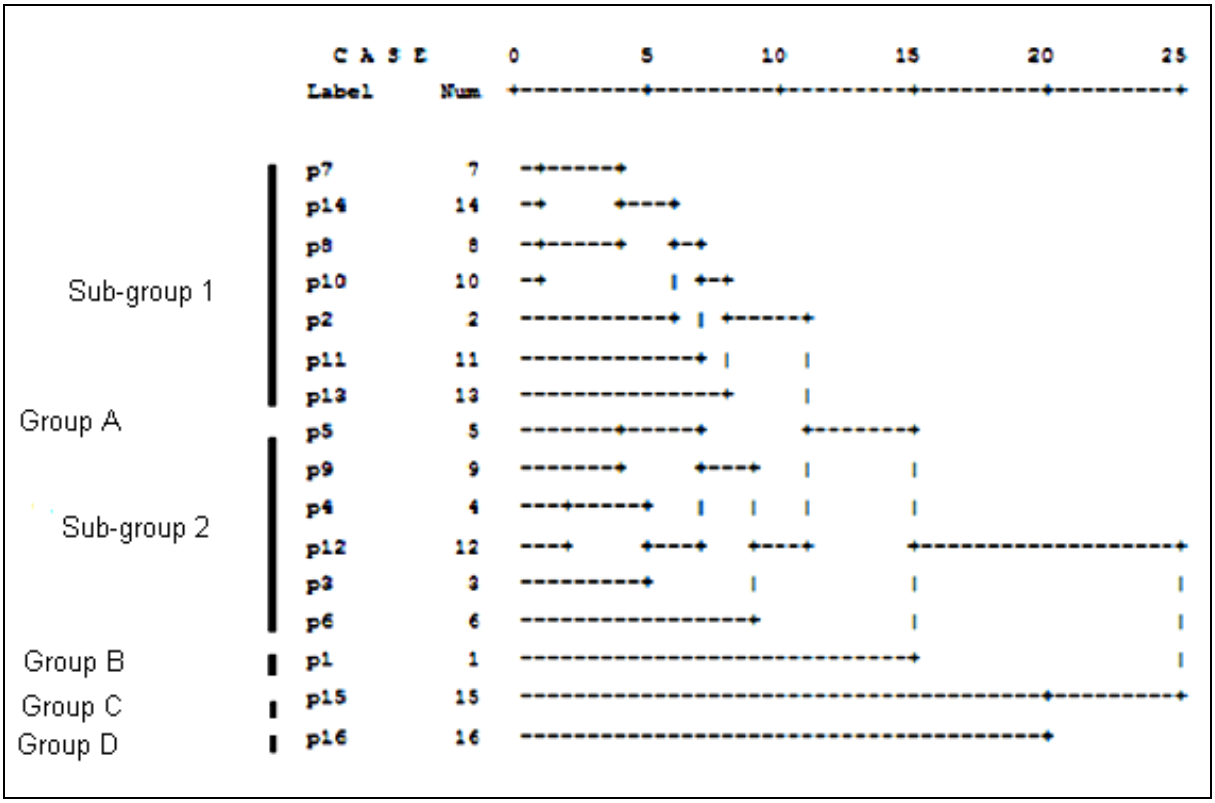

Figure 6. Dendrogram clustering revealed by 16 *Tunisian Calligonum* accessions using RAPD data.

(4.93 and 4.1) respectively.

Analysis of groups and establishment of the dendrogramm likeness

The composition of clusters obtained using RAPD markers has revealed similar groupings in some groups. The dendrogram obtained indicates four main groups (Figure 6).

The group A: composed of 13 accessions of *Calligonum* regrouped in two subgroups G1 and G2.

The sub-group G1: composed of accessions (P7, P14, P8, P10, P2, P11 and P13) and that are respectively (*C.azel* Jbil, *C.azel Kamour*, *C.comosum* Oued Jnein, *C.comosum* Lybia, *C.azel* ElBorma, *C.azel* Tiert cultivated in Arid Regions Institute of Medenine and *C.comosum* ElBorma). These accessions present the same profile of migration. The majority belongs to the Great Erg Oriental of the Tunisian south except P8 that belongs to the assembly-line of the Dhahar.

The subgroup G2: is represented by the accessions (P5 - P9 - P4 - P12 - P3 and P6) and that are respectively (*C.comosum* ElOuera, *C. comosum* Jbil, *C.azel* Tiert, *C.comosum* Tiert cultivated in Arid Regions Institute of Medenine, *C.comosum* Tiert and *C.comosum* Oued Dhabaa).

The group B: understands the accession P1 that represents (*C.arich* ElBorma) that is original of the Big Erg Oriental.

The group C: Is composed of the accession P16 that represents (*C.comosum*) descended of Oued Zridib of the Dhahar that characterizes itself by the absence of bands amplified by primers AF14 and AX16.

The group D: Is composed of the accession P15 that represents only (*C.comosum*) descended of the region of Ksar Ghilane and that characterizes itself by the absence of bands amplified by primers AF14 and AX16.

The genomic DNA of the 16 studied genotypes of the *Calligonum* kind has been amplified with these four selected primers. The 46 bands that represent the product of the amplification have been marked by numbers for their presence (1) or absence (0). Of these 46 bands, 41 are the polymorphous and 5 are monomorphics. Six among the 46 are some unique bands. It gives an average of 8.9 of polymorphism, 0.5 monomorphisms and 0.6 unique fragments by primer. The polymorphous strip percentage among the amplified bands showed a minimum of 75.0% for the primer (AF14) until a maximum of 100% for primers (A1 and A9) with

an average of 89.06% of polymorphism (Table 3).

The number of fragments amplified by sample is 8 for AF14 to 16 for AX16 (Figures 4 and 5). The dimension of fragments varies between 170 and 1300 bp. A dendrogramm based on the analysis by the method of Dice (RAPD) groups the 16 accessions into four main groups (Figure 6). One of the groups gathers 13 region samples, some of which are: Jbil, Kamour, Oued Jnein, Lybie, El Borma, and ElOuera cultivated at Tiert in the arid regions Institute of Medenine. This group can be subdivided into 2 sub-groups. The first subcluster (Ia) has seven samples, the second subcluster (Ib) consists of 6 samples. The second group presents only one sample of the zone of El Borma and it appears to be distinct from all others. The third group is formed by only one sample of Oued Zridib (Tunisian Dhahar). The fourth group is formed of a sample of Ksar Ghilane. While referring to the dendrogramm, we notice that accessions collected of the same region show an important rate of similarity; for example, P7 (*C.azel* Jbil) and P14 (*C.azel* Kamour) present a percentage of similarity of 95.7%. Accessions P3 (*C.comosum* Tiert) and P4 (*C.azel* Tiert) have a similarity of 85.70%. Accessions P15 (*C.comosum* Ksar Ghilane) and P16 (*C.comosum* Oued Zridib) have a similarity of 60%. However, accessions collected geographically of two distant regions showed a weak rate of similarity; for example, P8 (*C.comosum* Oued Jnein) and (P15 (*C.comosum* Ksar Ghilane) have a percentage of similarity of 53.3%.

ACP analysis

The ACP is a multivariate analysis founded on inter-relationships to transform variables of departure (in our case the RAPD markers) in independent synthetic variables or axes. This analysis permits to define markers that contribute best to the description of the structuring of the variability thus and to get a graphic representation of the individual projection according to their resemblance in the plan of axes of the ACP.

The matrix of data (number of bands revealed by primer/accession) has been treated by an analysis in main components (ACP) using the software SPSS. We notice that the first two components absorb 51.58% of the total inertia whereas the first three components absorb 64.15% of the total inertia. These results showed that RAPD marker types can be efficient in the description of the variability at these accessions. The own value, the variability (%) as well as the accumulated proportions, that define every axis, are represented in the Figure 3.

The projection of accessions in the definite plan by axes 1 and 2 is defined in Figure 7. The main components gather accessions in five groups. The first is constituted by p9 that detaches itself of the individual groups, particularly in relation to the axis 2 defined by the majority of variables. The second group is formed by p4, p5 and p12. The third group is composed of p1, p3 and p6. The fourth group is formed by p2, p7, p8, p10, p11, p13 and p14. In short, the fifth group, correlates negatively to the axis 1, united p15 and p16.

The projection of accessions in the definite plan by axes 1 and 3 (Figure 8), leads to results that are more or less similar to those of the dendrogramm. The main components gather accessions in five groups. The first is constituted by p6 and p12. The second group is formed by p2, p3, p4, p7, p8, p10, p11, p13 and p14. The third group is composed of p5 and p9. The fourth group is formed by p1, that detaches itself of the individual groups, particularly in relation to the axis 2 defined by the majority of variables. Finally, the fifth group unites p15 and p16.

DISCUSSION

The molecular markers always constitute a very important tool for the survey of the genetic diversity and detect interesting scorers correlated to ecophysiologic and agronomic characters of interests (Ghariani et al., 2003). They are ideal for the distinction between genotypes that are genetically similar to the use of an important number of primers (Zehdi et al., 2004; Meszaros et al., 2007). The dendrogram clustering revealed by the *Calligonum* accessions using RAPD data showed that the minimal distance gotten by the coefficient of similarity is 0.05 between P7 and P8 while the maximal distance is 0.74 between P1 and P15. These results showed that the geographical and ecological distribution of *Calligonum* accessions contributes to the genetic variability. According to Singh and Wadhwani, (1996), differences observed in the evolution of varieties in the distinct climatic zones are explained by differences in the response of every variety to the pressure of the selection in their natural environment. Therefore the level of polymorphism between the 16 genotypes of Tunisian *Calligonum* revealed by the RAPD markers remains logical. According to Bewal and Sharma, (2008), the certain genotype resemblances gotten by the RAPD dendrogramm, can reflect the morphological variation of accessions. Mabberely (1990) showed that *Calligonum*, as one of the big genera in Polygonaceae with approximately 80 species, represent a rapid diversification for a short time in hot and arid deserts of Western Central Asia.

Morphologically, P1 (*C.arich*) collected of El Borma showed the maximal variation with regard to the height of the plant, the branch colors, inter-nodes length, the flower color and the color and the shape of the fruit. Populations collected of the Dhahar zone and ElOuera zone arehete-rogeneous. However, populations of the Great Erg Oriental appear homogeneous with a very limited morphological and cytological diversity that is recorded by (Dhief et al., 2009). According to Sage (2004) and

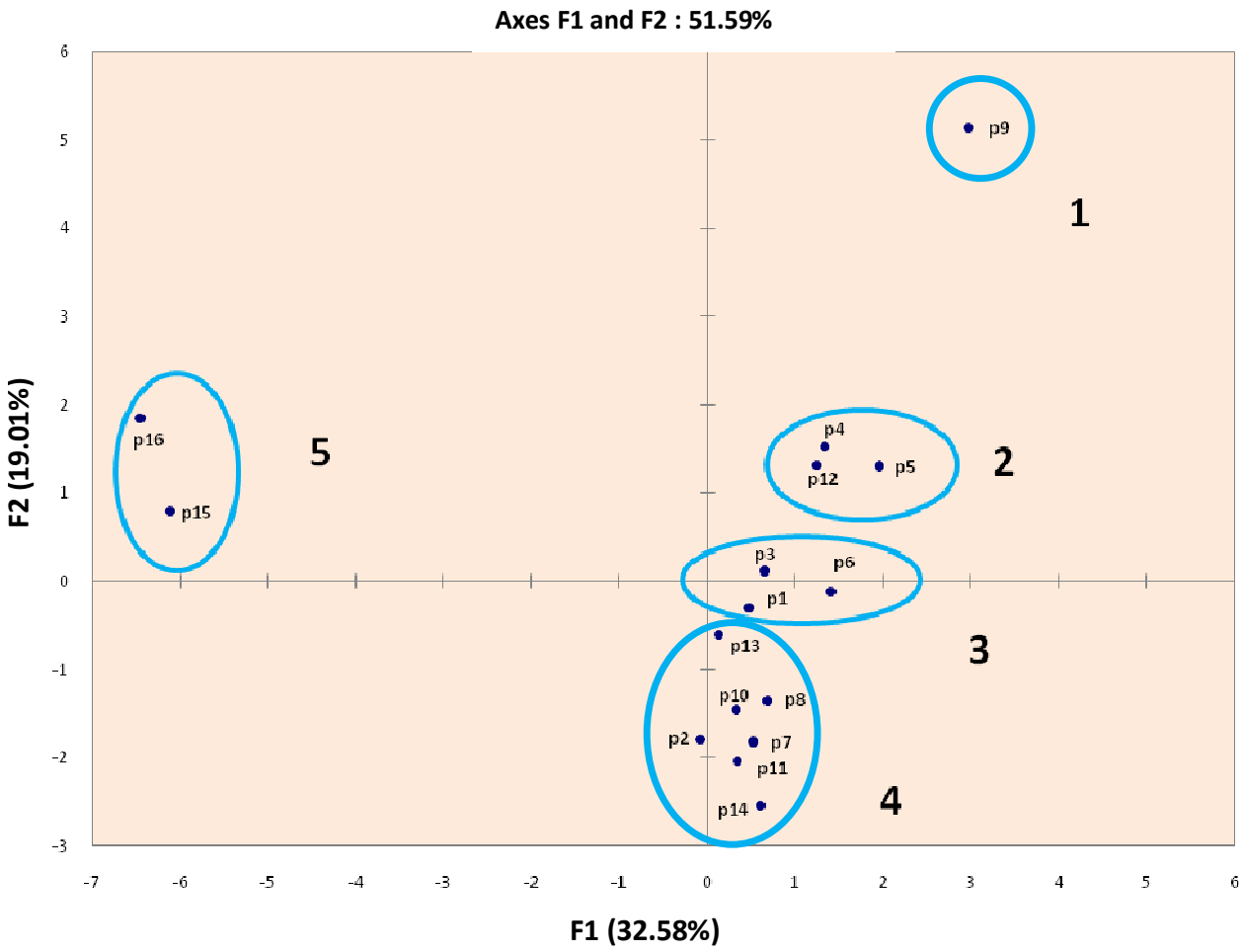

Figure 7. Graphic representation of the spatial distribution of the *Calligonum* accessions in plans (1-2) of an analysis in main (ACP) and (SPSS) components, achieved from the RAPD markers

Pyankov et al. (2000), *Calligonum* diversification may be caused by several factors including C4 photosynthetic pathway, bristled/winged fruits a dispersal units and hybridization/introgression followed by tetraploidy.

Unusually, P15 and P16 are grouped far away from the other samples. They are individuals that generallypresent a straight trunk characterized by green branches of 60 cm length average. Pervinquiere (1912) showed that the Ksar Ghilane area was occupied by a kind of *Calligonum* known as "*Artha*" which has a branched port and submerged in the most part in the sand. So the disappearance of this species "*Artha*" and the appearance of a species with a right port and long leaves may be due to natural selection exerted on the first kind for the second or the effect of grazing. According to Loarce et al. (1996), this observation can be assigned to the mistake of the scorer molecular chosen or to the mistake of the polymorphism level detected and can be reinforced again by the important number of loci and their large genomic cover what permits the oreliable assessment obtained in the genetic relations between accessions. *C. arich* collected of El Borma gives several specific bands that can be sent back to the morphological and cytological observations of this plant. This sample has some red flowers and red and purple fruit that give a reddish appearance during flowering and fruition. El Borma site is a known desert region by its sandy and slightly acidic soil. Kweon et al. (2001) showed that the geographical and climatic interruption by the Heng-duanshan mountains may have caused the genetic divergence in *Fagopyrum cymosum*. Results gotten from samples of this region showed a particular genetic variation (the expression of a certain level of genomic DNA by plants) that can be a demonstration of the response or the tolerance of the species to the biotic and abiotic stress. Tavakkoli (2008) showed that the Correlation between geography and morphology and the lack of correlation between genetic markers and either of these 2 parameters imply that the differences between populations may be the result of environmental differences. The dendrogramm represented by it (Figure 6) showed a grouping according to the geographic place of

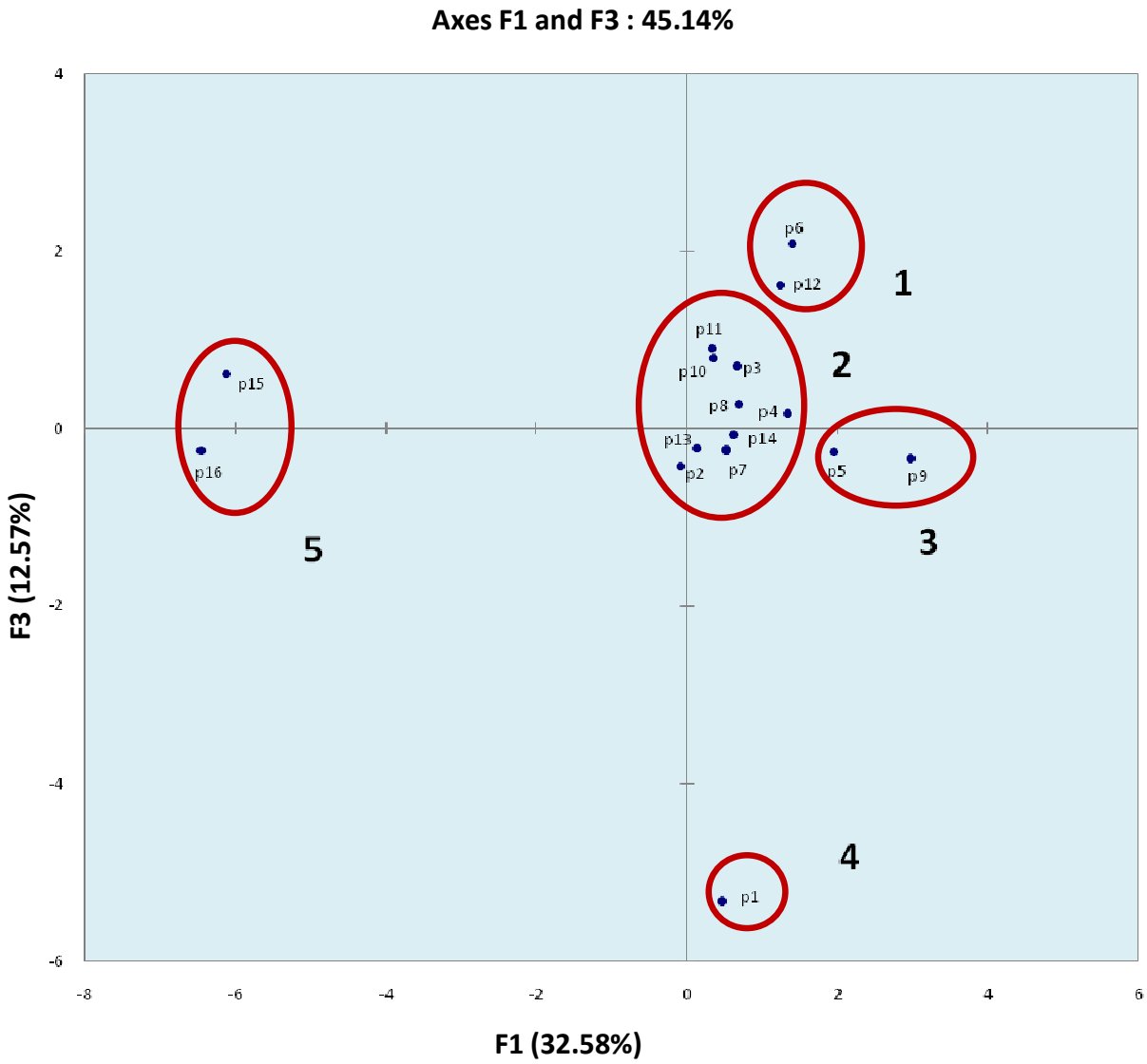

Figure 8. Graphic representation of the spatial distribution of the *Calligonum* accessions in plans (1-3) of an analysis in main (ACP) and (SPSS) components, achieved from the RAPD markers.

species collected. Accessions collected of the same region showed an important rate of similarity, whereas accessions collected of geographically distant regions showed a weak rate of similarity. The ACP analysis showed the existence of *Calligonum azel* accessions and *Calligonum comosum* accessions in the same group (p2, p3, p4, p7, p8, p10, p11, p13 and p14). This can explain why *Calligonum comosum* accessions are arisen from *Calligonum azel* accessions or why they have the same ancestor. Ferchichi (1997) showed that Tunisian *Calligonum comosum* (Kamour accession) with 2n = 36 is arisen from Tunisian *Calligonum azel* (Kamour accession) with 2n = 18. He also shows that *Calligonum comosum* has a genetic elasticity caused by the polyploidy. According to Kweon et al. (2001) the tetraploid populations of *F. cymosum* have arisen allopatrically from a diploid progenitor at least twice, once in the Tibet-Himalayan area and once in the Yunnan-Sichuan area. Stehlik and Blattner (2004) showed that the patterns of inter-specific and intra-specific variation found in *Rumex* genus (Polygonaceae) are in accordance with proposed modes of the evolution of sex chromosomes. Such a similarity is the indication of the same common ancestor existence between *Calligonum arich*, *Calligonum azel* and *Calligonum comosum* and the weak divergence is owed to mutations generated by the new climato edapphic conditions. Raju and Raju, (2001) signal that species with crossed fertilization (by anemophilia), as for many species of the family of the Polygonaceae, showed some morphological, cytological and molecular variations even, without studying the outdistance factors that separate the different studied species. The accession P1 (*C.*

arich) collected of tops of dunes of the region of El Borma and the accession P15 (*C.comosum*) collected of the Souframanien et al. (2004) signals that it is possible that the most distinct DNA profiles generally contain an elevated number of new alleles. Ren and Tao (2004) using randomly amplified polymorphic DNA (RAPD) analyses of 14 Chinese *Calligonum* species showed that *C. junceum* is positioned far from the remaining species studied and species having bristled fruit (sect. *Calligonum*) are not grouped in a single cluster as the winged fruit species (sect. *Pterococcus*). These accessions permit to discover the biggest number of potentially useful alleles as of genes of resistance or tolerance to stress. Runo and Muluvi, (2004) used some RAPD markers to discover the inter- and intra- genetic diversity populations of *Melia volkensii* to preserve them and to find the most suitable management strategy. Plants of the same group have a tendency to survive together in the same region. There are some accessions of *Calligonum* that are form some specific groups and they can be assigned to the different conditions of their life surroundings. Outside, for some non comprehensive reasons, the genetic variability between accessions of *Calligonum* is due to the occupied different specific geographical places, and therefore to their capacities to adjust to the specific climatic conditions. The survey of germplasms of the Turk grape suggested that the geographical and ecological distribution of plants contributes to a high genetic variability (Karatas and Agaoglu, 2008). According to Liu and Pei, (1999) and Runo et al. (2004), the similar strips gotten by RAPD from different individuals are not necessarily homologous, although, they can share the same dimension in pairs of basis. This situation can play a main role in the genetic report calculation between accessions. The present survey is a tentative that looks for the interrelationship between the inter- and intra-specific genetic variation in the *Calligonum* genus of Tunisia and the geographical distribution of species of this kind. According to Latha and Thiyagarajan, (2004) and Santallas and Power, (1998), the simple hybridization by the use of polymorphism primers can confirm the size of the intra and inter-specific genetic variation and can give a supplementary result in the genetic report between the different accessions. The big likeness observed between *C.azel* and *C.comosum* species, explains their common ancestor. Genetic diversity among population levels of genetic variation within rare or geographically restricted plant species are typically low (Hickey et al., 1991), although, several recent studies have indicated high levels of genetic variability in some rare species (Archibald et al., 2001). However, the divergence observed between *C. arich* species and the other species of *C. azel* and *C.comosum*, leads to the thought that those species would be able to be far to the origin of a common ancestor. Frye and Kron (2003) and Kim and Donoghue (2008a) showed molecular phylogeny of *Persicaria* where two species of *Polygonum* appeared with strongly supported relationship. Presence of two accession of each of *P. glabra* and *P. hydropiper* in the *Polygonum* cluster indicated that these *Persicaria* species shared common ancestor with *Polygonum*. These species (*Calligonum arich*, *Calligonum azel* and *Calligonum comosum*) could be therefore either different species or that they are at the level coins related species (they have the same ancestor) in the time following to the simple mutations of their genome. Tavakkli et al. (2008) showed that if populations and accessions remain distinct under common environmental conditions, then it is clear that there is a selection occurring among these populations and that they merit conservation status. Nevertheless, this study showed the existence of a natural genetic diversity between *Calligonum* species collected of different regions of Tunisia. This diversity is formulated according to the molecular analysis of the following form: in Tunisia we can find the *Calligonum arich* species in the dune crests, of the Great Erg Oriental distinguished by red flowers and an altitude that sometimes overtakes 10 m, *Calligonum azel* on slope dunes of the Great Erg Oriental, *Calligonum polygonoides* Ssp.*comosum* in the inter-dunes generally characterized by a round trunk with green branches of 50 cm length average and with the existence of another new species or or subspecies of *Calligonum* that is the one of Ksar Ghilane. It is probably a new *Calligonum polygonoides* subspecies or another shape of *Calligonum comosum* that is an unpublished species in the flora of Tunisia adapted to the new biotic and abiotic conditions installed in this zone. This new shape of *Calligonum* presenting a straight trunk with green branches of 60 cm length average and exist generally in the Geat Erg Oriental limits, in the Tunisian Dhahar zone and even on the road side. The resolution of this problem requires the other researches as the sequencing of DNA of every species. The big likeness observed Tavakkli and al. (2008) showed that one accession of *Rumex chalepensis* is in full confidence with two accessions of *Rumex dentatus*, while second accession (13006) is distant from the first one, showing value of 50% bootstrap with *Rumex dentatus* and first accession of *R .chalepensis*. This special position of one accession of *R. chalepensis* may perhaps be its misidentification so that it represents another *Rumex* species or a hybridization product with a *R. dentatus*. These species of *Rumex* are identified by extensive branching in the upper half, basal leaves 2-3 times longer than broad, valves with 4 to 9 unequal teeth near to the base in *R. chalepensis* while *R. dentatus* with branching near to the base, panduriform leaves and 3 to 4 teeth at each margin of the valves (Rechinger, 2001).

This study is the first one to report the use of the RAPD marker system for determining relationships, at inter- and intra-specific level in the Tunisian *Calligonum* genus (Polygonaceae). This helps us to preserve, valorize and find appropriate strategy for these species to use in the

future rehabilitation programs of Tunisian degraded zones.

Conclusion

This study is an attempt that searches the correlation between the inter- and intra-specific genetic variation in the Tunisian *Calligonum* genus and the geographical distribution of these species of this genre. The genetic variability between accessions of *Calligonum* is due to different occupied and specific geographical places, therefore, their capacity to adapt to specific edapho-climatic conditions. The accession P1 (*Calligonum arich*) is collected from the top dunes of El Borma and the accession P15 (*Calligonum polygonoides Ssp. Comosum*) collected from Ksar Ghilane region, formed some distinct DNA profiles. The dendrogramm shows a groupment according to the collection area of these species. This intra- and inter-specific genetic variation gives a supplementary result in the genetic link between the different accessions that help to easily distinguish between the individuals of this genre.

In Tunisia, we can find *Calligonum arich* species in the top of big dunes distinguished by its red flowers and its altitude overtaking sometimes the 10m, *Calligonum azel* in the slope of the big dunes, *Calligonum polygonoides Ssp. Comosum* in the inter-dunes characterized generally by a round and dense trunk with green branches that do not overtake the 50 cm length average average and with the existence of another new species or subspecies of *Calligonum* that isthe one of Ksar Ghilane. It is probably a new *Calligonum polygonoides* subspecies or another shape of *Calligonum comosum* that is an unpublished species in the flora of Tunisia adapted to the new biotic and abiotic conditions installed in this zone. This new shape of *Calligonum* presenting a straight trunk with green branches of 60 cm length average and exist generally in the Geat Erg Oriental limits, in the Tunisian Dhahar zone and even on the road side between all *C. azel* and *C. comosum* species, explains their common ancestor. However, the divergence observed between the *C. arich* species and the all other species of *C. azel* and *C.comosum*, leads to think that those species would be able to be far from the origin of a common ancestor. These species - *C. arich, C. azel* and *C.comosum* -could therefore be either different species or that they are at the level coins related species in the time following to the simple mutations of their genome.

It may be that although all Tunisian *Calligonum* accessions are more or less similar, they are distinct from the remainder of the species, and these limited accessions in Tunisia desert merit attention. Likewise, even if the Tunisian *Calligonum* accessions do merit a taxonomic status, their geographic location at the Southern limits of the species range implies that loci or alleles may be present and should be accounted for conservation programs.

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