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Molecular Systematic Study Of The Genus *Fagonia* L. In Libya

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ABSTRACT

Molecular analyses of ten *Fagonia* species grown widely in the Libyan Desert have been carried to investigate the taxonomic relationship between them and to evaluate the genetic distances among them. To achieve our aim RAPD technique carried out through six arbitrary primers. Comparing with ladder DNA marker, the obtained data were computerized and analyzed using SYSTAT program. The studied species are *F. arabica* L., *F. bruguieri* DC, *F. cretica* L., *F.glutinosa* Delile, *F. indica* Burm., *F. microphylla* Pomel, *F. sinaica* Boiss, *F. schweinfurthii* Hadidi, *F. tenuifolia* Steud. and *F.thebaica* Boiss. The genetic variability among the ten *Fagonia* species estimated using the DNA protein sequencing obtained from primer 1, indicates that *F. indica* and *F. glutinosa* are very closely related while *F.cretica*, *F.microphylla* and *F.arabica* related to each other and gathered together in another group. The dendrograms of the six primers via UPGMA method according to RAPD finger printing gave two clusters with homology percentage 9%. The first one has *F.microphylla* and *F.schweinfurthii* at 50% similarity index. The second cluster divided into two sub-clusters. The first one included three *Fagonia* species (*F.cretica; F.indica* and *F.glutinosa*). The second sub-cluster subdivided into two other sub-clusters. The first one contained *F.arabica* and *F.bruguieri* at 50% similarity index. The other sub-cluster gathered *F.sinaica* and *F.thebaica* and, both species in genetic relationship with *F.tenuifolia*.

Key words: DNA sequencing - *Fagonia*- Libya- Molecular analyses- Taxonomy- RAPD.

1. INTRODUCTION

Genus *Fagonia* is a member of Zygophyllaceae, subfamily Zygophiloideae which is included in the eurosid I clade (APG III, 2009). The genus comprises Thirty-five species all over the world (Plant of the World, 2018). In Libya, there are only ten species growing in different phyto-geographical regions (Abdul Ghafoor, 1977). *Fagonia* species are generally spiked under-hedge, erect herbs, some species covered by glandular hairs, branches malign, stem terete, striate and glabrous. Leaves simple or compound, 1-3 foliate; petioles from 3 - 30 mm long, profoundly striate, extremely thin; stipules 2 sets of sharp thin thistles (Farheen et al, 2015; Puri and Bhandari,

2014, Taia et al., 2015). The taxonomy of the genus is very difficult mainly due to a high degree of phenotypic plasticity and adaptations to climatic conditions (Zohary 1972; Danin 1996). Accordingly the taxonomy of the genus has been faced with many proposals. From the most important work in the taxonomy of the genus is that done by Ozenda and Quézel (1956) who grouped the North African *Fagonia* species into four natural groups, which can be considered as sections according to Melbourne System of Nomenclature (2012). This division based mainly on vegetative morphological characters, the Quezel four groups of *Fagonia* is (1) *F. kahirina-cretica*-flamandii, (2) *F. arabica-bruguieri*, (3) *F. glutinosa-latifolia*, and (4) *F. microphylla*. Also, the works done by El Hadidi (1966) and Batanouny and Batanouny (1970) add another contribution to the taxonomy of the genus. They grouped the 18 Egyptian species of *Fagonia* into three groups based on anatomical structures; they constructed an artificial key for their identification based on both morphological and anatomical characters. Taia et al. (2015, 2016, 2017 & 2021) studied the Libyan species in different aspects to clarify the relationships between them. They found that the morphological and floral characters can be of use in solving the taxonomical confussion between the species.

After all the studies done, the delimitation of species in *Fagonia* is still in need to more investigations for being notoriously difficult and confusing. This is caused by the great variation in most morphological characters within many species and between individuals of the same species grown in different habitats. The first complete modern treatment of the genus has been done by (Beier, 2005). His work was mainly concerning the geographical distribution of the African species, their center of origin and distribution. According to this revision, *Fagonia* considered a genus of 34 species, distributed mainly in warm and arid areas all over the world, except Australia, with great diversity of species in the Horn of Africa region and Baja California. Genus *Fagonia* is one of the critical genera of the Zygophyllaceae family, as mentioned. Many species are very closely allied and are linked by intermediate forms, which make a species delimitation rather difficult. Previous works on the genus based mainly on vegetative, floral and anatomical characters. Palynological investigations are few and when done it did not give important suggestion for the division of the genus, For that, this study carried to clarify the taxonomical relationship within the *Fagonia* species grown in Libyan desert by molecular analysis.

2. MATERIALS AND METHODS

Ten species belonging to genus *Fagonia* (*F.arabica*L., *F.bruguieri*DC., *F.cretica*L., *F.glutinosa*Delile., *F.indica*Burm, *F.schweinfurthii*Hadidi, *F.sinaica*L., *F. microphylla*, *F.tenuifolia*Steud., *and F.thebaica*Boiss.) were subjected in this study. These species grown mainly in sandy or gravelly habitats and tolerate with soil salinity. The specimens collected through field trips during 2014 till 2017, to different locations covering most of the habitats in Libya. The study area extends from the eastern plains (Al-Gabal Al-Akhdar) in the east to GabalNaffusah (Nalut and Ghadames) in the west to Sebha and El-Kufra in south, the name of 20 visited locations as shown in (Table1) (Map 1) during the period from 2014 till 2017. Ten individuals from each species have been collected for herbarium preparations and allocated at EL-

Mergib university herbaria. Leaves from each species were gathered, put in paper bags for the molecular analyses.

Map 1. A sataliate google earth map showing detailed sector and the distribution of the diffreent locations in the study area in Libya (■ and arrows: Locations, ●: Cities)

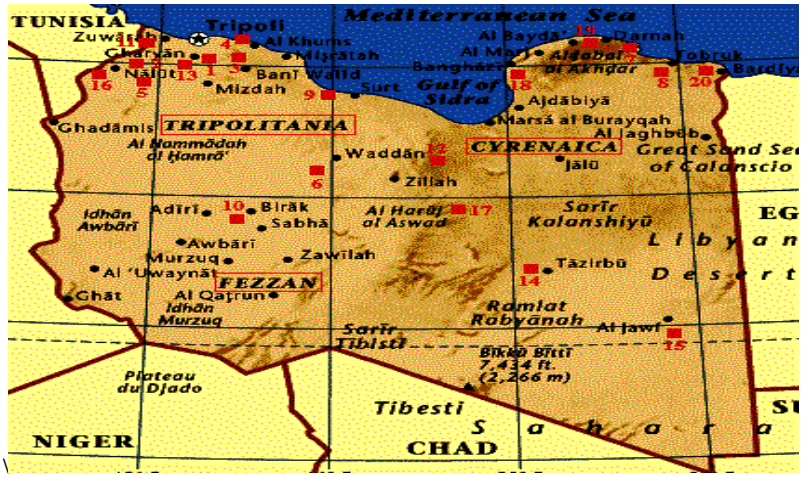


Table 1. Name of 20 locations of the collected *Fagonia* species in Libya.

No	Location	Region	No	Location	Region
1	Gharian ,Gebel Nafousa	West	11	Wadi El-Aital	west
2	Giado,GebelNafousa	West	12	Sokna	Middle
3	Mesallata	west	13	Tagrenna, Jefren	West
4	Alkhums	west	14	Tazerbo	South
5	WadiMalah, Nalut	west	15	Gebel Uwainat	South
6	Hun	Middle	16	Wazen	West
7	WadiDerna	East	17	El-Soda mountain	South
8	Tobruk	East	18	Benghazi, Teka	East
9	Weshka	Middle	19	Al-Abidaa, Shahat, Ras El-Hellal	East
10	Brak, Sebha	South	20	Musaid	East

- **Extraction of genomic DNA**

Genomic DNAs of the ten *Fagonia* species were prepared according to Omega Co. (USA.LMt.) manufacturer protocol as the following:

For each 100 mg leaf powders, 550 µL of lysis buffer solution were added; shacked gently; incubated for 30 min on ice; centrifuged at 1200 rpm for 10 min at 4°C and the supernatant was removed. The pellet was re-suspended in 1 ml of lysis buffer; centrifuged at 1200 rpm for 10 min at 4°C and the supernatant was removed. The pellet was resuspended in 0.5 ml SE-buffer and centrifuged at 1200 rpm for 10 minutes at 4°C. The supernatant was removed and the pellet was resuspended in 1 ml SE-buffer. 40 µl proteinase K (10 mg/ml) and 250 µl 20% SDS were added; shaked gently and incubated overnight at 37°C in a water bath. Then, 5 ml SE-buffer and 10 ml

phenol were added; shaked by hand for 10 min and centrifuged at 3000 rpm for 5 min at 10°C. The supernatant was transferred into a new tube; 1 ml phenol/chloroform/ isoamyl alcohol (25:24:1) was added; shaked by hand for 10 min and centrifuged at 3000 rpm for 5 min at 10°C. The supernatant was transferred again into a new tube; 1 ml chloroform/ isoamyl alcohol (24:1) was added; shake by hand for 10 min and centrifuged at 3000 rpm for 5 min at 10°C. The supernatant was transferred into a new tube; 100 µl of 3 M sodium acetate (pH 5.2) and 10 ml isopropanol were added and shaked gently until the DNA precipitated. Finally, the DNA was captured and transferred into a new tube by a glass pipette; washed with 70% ethanol; dissolved in 0.1 ml TE-buffer (10mM Tris-HCl and 1mM EDTA-pH8) overnight at 4°C on a rotating shaker and stored at the same temperature until use.

- **Random Amplifid Polymorphic DNA (RAPD) procedure**

Ready-To-Go RAPD Analysis Beads (GE Healthcare Life Sciences, 27-9502-01, with primers) was used for the Random Amplified Polymorphic DNA (RAPD) technique according to manufacturer protocol as the following:

- **RAPD -PCR amplification**

Using six arbitrary 10 mer primers Table (2), the total genomic DNAs were amplified through GeneAmpPolymerase Chain Reaction (PCR) system cycler. The PCR reaction components are shown in Table (3). The PCR program was performed in a thermal cycler programmed at 40 cycles. Each cycle consisted of denaturation at 93°C for 30 sec followed by annealing at 51°C for 30 sec and extension at 72°C for 30 sec. There was an initial delay for 15 min at 95°C at the beginning of the first cycle and 10 min delay at 72°C in the end of the last cycle as a post extension step. The product was stored at –20 or 4°C.

Table 2. Arbitrary primers used for Fagonia genomic DNAs amplifications

Number of primer	Nucleotide sequence (5′- 3′)
1	GGTGCGGGAA
2	GTTTCGCTCC
3	GTAGACCCGT
4	AAGAGCCCGT
5	AACGCGCAAC
6	CCCGTCAGCA

Table 3. Master Mix components for PCR reactions

Master Mix component	Amount	Final concentration
Sterile nuclease free water	17.8 µl	
10x Taq buffer	2.5 µl	1.0 x
4mM PCR nucleotide mix	2.5 µl	0.2 mM
Primer (5pmol /µl)	1.0 µl	5.0 pmol
Taq DNA polymerase (5u /µl)	0.2 µl	1.0 u
DNA extracted sample (50ng /µl)	1.0 µl	25.0 ng
Total	**25.0 µl**	

- **Agarose gel electrophoresis and detection of the amplified products**

5 µl of each PCR products were separated on agarose gel electrophoresis using 1.5% (w/v) agarose in 0.5x TBE buffer. Electrophoresis was performed at 80 Volt for 100 min in 0.5x TBE buffer (50 ml of 10X TBE stock solution {Tris-base (108g); Boric acid (55g) and EDTA -pH 8 (9.3g) in 1 L distilled H_2O} was added to 950 ml distilled H_2O) as running buffer. Then, the gel was stained with 0.5µg/ cm^3 (w/v) ethidium bromide solution for 30 min followed by 20 min destain in distilled water. Finally, the gel was photographed by gel documentation system and the lengthof each band was estimated usingthe DNA ladder marker.

- **Data analysis**

Gel documentation system (Geldoc-it, UVP, England) was applied for data analysis using Totallab analysis software, ww.totallab.com, (Ver.1.0.1).

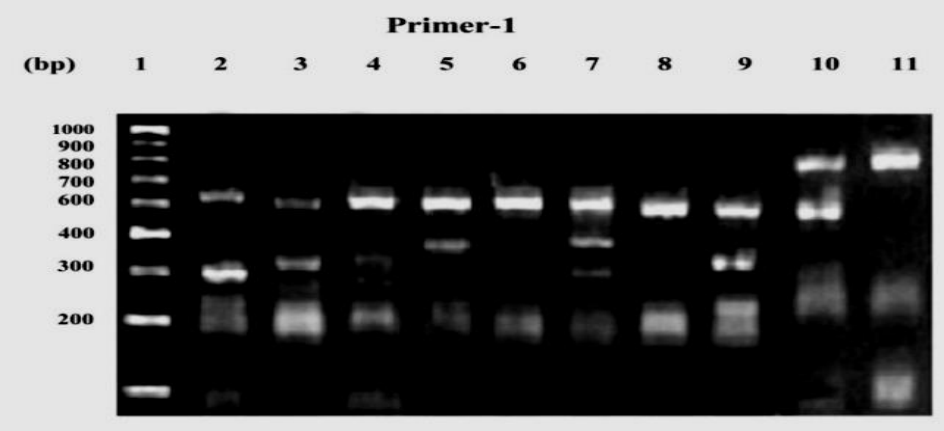
3. RESULTS

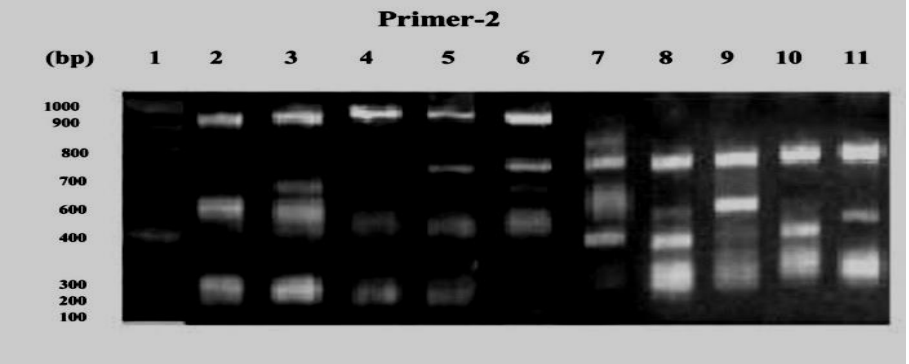
Random amplified polymorphic DNA (RAPD) analysis:

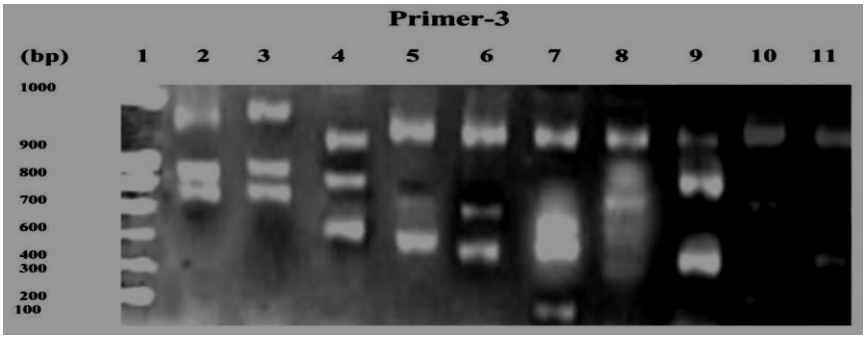
In order to evaluate the genetic distances among the ten*Fagonia* species understudy, RAPD technique was employed through six arbitrary primers. Comparing with ladder DNA marker, the obtained data were computerized and analyzed.

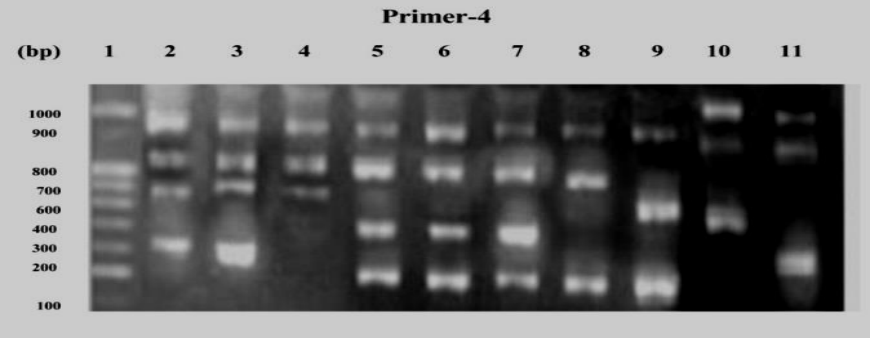
For each primer, the numbers and lengths of the reproducible fragments varied from species to another (Figure 1 and Tables 4). The maximum number of the amplified bands (9) was recorded for *Fagonia thebaica* with primer-5. The DNA lengths of these fragments ranged from 1030.769 to 88.000bp. On the contrary, primers 1 and 2 amplified only 2 bands for *F. sinaica* (769.884 and 306.473bp) and *F. cretica* (339.142 and 149.588bp). The largest DNA fragment lengh (1033.333bp) was recorded for *F.tenuifolia* with primer-4 and the lowest one (76.000bp) was for *F.sinaica* with primer-5.

Also, the computraized RAPD data revealed that, the amplified fragments ranged from 34 for primer-3 to 62 for primer-5 with total of 268 reproducible fragments (Table 5 and Figure 2). The polymorphic bands were 107 (39.9% polymorphism). The minimum number of the polymorphic fragments (10) was indicated for primer-2 with polymorphism percentage 25.6%. In contrast, primer-5 exhibited the maximum number of polymorphic bands (41) and polymorphism (66.1%). Intermediate valus were illustrated with the other primers. The polymorphic fragments for primers-1; 3; 4 and 6 were 13; 14; 15 and 14 with 34.2; 41.1; 35.7 and 26.4% polymorphism, respectively.


Primer-1
(bp) 1 2 3 4 5 6 7 8 9 10 11
1000
900
800
700
600
400
300
200


Primer-2
(bp) 1 2 3 4 5 6 7 8 9 10 11
1000
900
800
700
600
400
300
200
100


Primer-3
(bp) 1 2 3 4 5 6 7 8 9 10 11
1000
900
800
700
600
400
300
200
100


Primer-4
(bp) 1 2 3 4 5 6 7 8 9 10 11
1000
900
800
700
600
400
300
200
100

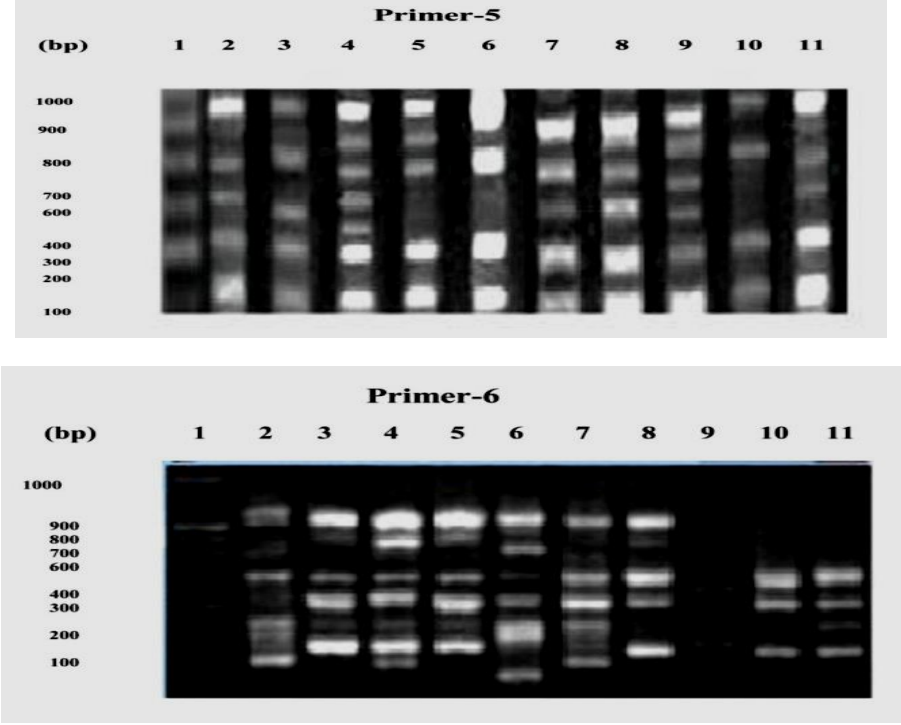


Figure1. Photographs illustrating RAPD products for ten *Fagonia* species using six randomly primers. Lane 1: ladder DNA marker with fragments lengths in base pairs (bp); Lanes 2-11: *Fagonia arabica; Fagonia bruguieri; Fagonia cretica; Fagonia indica; Fagonia glutinosa; Fagonia microphylla; Fagonia sinaica; Fagonia schweinfurthii; Fagonia tenuifolia* and *Fagonia thebaica*, respectively.

Table4. Numbers and lengths of the reproducible fragments for ten *Fagonia* species using primers 1 to 6:

Primer-1

Species	Numbers of fragments	Fragments lengths (bp)
Fagonia arabica	5	816.496 – 28.571
F. bruguieri	4	784.604 – 305.649
F. cretica	5	808.088 – 335.282
F. indica	3	800.000 – 351.282
F. glutinosa	3	816.496 – 305.649
F. microphylla	4	808.088 – 314.608
F. sinaica	2	769.884 - 306.473
F. schweinfurthii	4	769.884 – 306.473
F. tenuifolia	4	1014. 286 – 553.291
F. thebaica	4	984.944 – 100.000
Total	**38**	

Primer-2

Species	Numbers of fragments	Fragments lengths (bp)
Fagonia arabica	6	970.327 – 139.980
F. bruguieri	4	536.609 – 139.980
F. cretica	2	339.142 – 149.588
F. indica	3	632.243 – 158.937
F. glutinosa	3	632.243 – 356.681
F. microphylla	4	818.413 – 290.597
F. sinaica	5	700.000 – 206.729
F. schweinfurthii	4	700.000 -218.640
F. tenuifolia	4	736.608 – 237.373
F. thebaica	4	725.542 – 228.750
Total	**39**	

Primer-3

Species	Numbers of fragments	Fragments lengths (bp)
Fagonia arabica	3	996.316 – 749.568
F. bruguieri	3	1000.000 – 736.673
F. cretica	3	967.981 – 619.900
F. indica	4	992.344 – 577.093
F. glutinosa	3	992.344 -446.694
F. microphylla	4	987.579 – 85.714
F. sinaica	4	987.579 – 676.136
F. schweinfurthii	3	971.840 -490.312
F. tenuifolia	4	975.452 -169.118
F. thebaica	3	957.452 -469.280
Total	**34**	

Primer-4

Species	Numbers of fragments	Fragments lengths (bp)
Fagonia arabica	4	936.145 – 411.427
F. bruguieri	5	936.145 – 343.964
F. cretica	4	936.145 – 630.555
F. indica	5	913.014 – 187.799
F. glutinosa	4	894.345 – 157.593
F. microphylla	4	906.233 – 166.681
F. sinaica	3	920.292 – 166.681
F. schweinfurthii	4	889.219 – 135.170
F. tenuifolia	4	1033.333 – 483.065
F. thebaica	5	981.009 – 242.313
Tolal	**42**	

Primer-5

Species	Numbers of fragments	Fragments lengths (bp)
Fagonia arabica	5	1000.000 – 119.951
F. bruguieri	5	1007.692 – 131.926
F. cretica	8	925.996 – 100.000
F. indica	5	982.627 – 100.000
F. glutinosa	7	1030.769 – 127.934
F. microphylla	6	857.248 – 88.000
F. sinaica	7	876.967 – 76.000
F. schweinfurthii	6	880.816 – 80.000
F. tenuifolia	4	982.627 – 139.914
F. thebaica	9	1030.769 – 88.000
Total	**62**	

Primer-6

Species	Numbers of fragments	Fragments length (bp)
Fagonia arabica	8	967.797 – 153.737
F. bruguieri	5	926.442 – 214.831
F. cretica	6	933.972 -189.473
F. indica	5	909.486 – 228.385
F. glutinosa	7	944.168 – 142.721
F. microphylla	7	922.445 – 153.737
F. sinaica	5	930.282 – 204.660
F. schweinfurthii	3	707.991 – 221.857
F. tenuifolia	3	522.397 -210.979
F. thebaica	4	555.991 – 202.379
Total	**53**	

Table5. Numbers of the amplified and polymorphic fragments as well as polymorphism percentage for ten *Fagonia* species using six randomly primers.

Primer	Total number of amplified fragment	Number of polymorphic fragment	Percentage of polymorphism (%)
1	38	13	34.2
2	39	10	25.6
3	34	14	41.1
4	42	15	35.7
5	62	41	66.1
6	53	14	26.4
Total	268	107	39.9

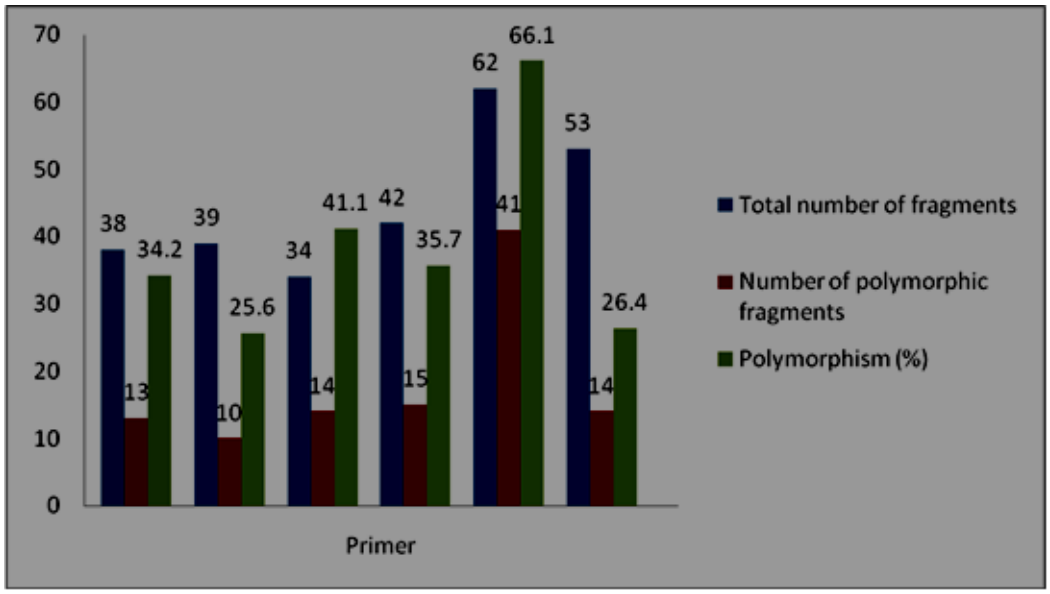


Figure2. Histogram illustrating the computerized RAPD results of ten *Fagonia* species using six arbitrary primers.

The phylogenetic relationships among *Fagonia*species baesd on RAPD data:

The genetic variability among the ten *Fagonia* species were estimated using the DNA fingerprinting data of each primer (Figure 3) and all primers (Figure 4) via UPGMA method.

The dendrogram based on primer-1 demonstrated two clusters with homogeneity < 5%. The first one contained only *F. bruguieri*. The second cluster divided into two subclusters with similarity 7%. The first subcluster grouped five species. The most related species were *F. indica* and *F. glutinosa* with homogeneity 69% and, both species had homology percentages 51; 37 and 29% with *F.cretica*, *F. microphylla* and *F. arabica*, respectively. The second subcluster consisted of four species. The similarity of *F. sinaica* and *F. schweinfurthii* was 69% as well as *F. tenuifolia* and *F. thebaica* had relative homogeneity 51%.

Two main clusters were also detected for primer-2 with homology percentage <10%. The first one included *F. microphylla; F. sinaica* and *F. schweinfurthii*. *F. sinaica* and *F. schweinfurthii* had homogeneity 67% and, both species were similar to *Fagonia microphylla* with 36%. The other cluster divided into two subclusters with homology percentage 11%. The first one included *F. arabica, F. bruguieri, F. cretica, F. indica* and *F. glutinosa*. The most related species were *F. cretica* and *F.indica* with homogeneity 81% and, both species had similarity 64 and 55% with *F.bruguieri* and *F. glutinosa*, respectively. All the four species had relative homogeneity 23% with *Fagonia arabica*. The second subcluster contained *F. tenuifolia* and *F.thebaica* with homology percentage 76%.

The dendrogram based on primer-3 exhibited two main clusters with similarity 5%. Each cluster divided into two groups forming finally four subclusters. The first subclusterhad

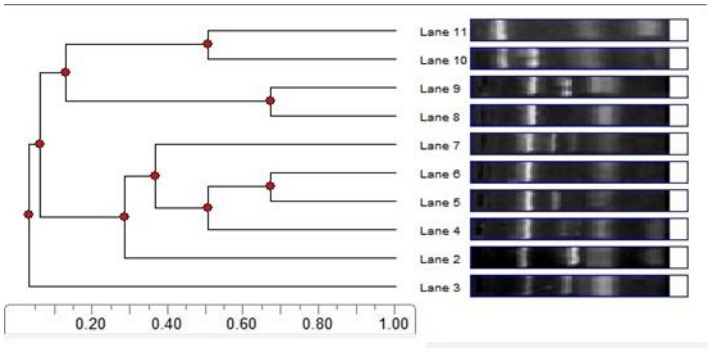
homogeneity 9% with the second one and included only *F. microphylla*. The second cluster grouped three species. *F. arabica* and *F.bruguieri* related to each other with homology percentage 34% and, both species similar to *F. sinaica* with 29%. The relative homogeneity betwee the third and fourth subclusters was 11%. The third subcluster consisted of *F. indica* and *F. glutinosa* with similarity 59%. The three species *F. cretica, F. schweinfurthii* and *F. thebaica* of the fourth subcluster had homology percentage 35% and, were similar to *F. tenuifolia* with 25%.

Three clusters were detected for primer-4. The first one divided into two subclusters with homogeniety 23%. The first subcluster included *F. bruguieri* and *F. cretica* with homology percentage 72% and, both species were similar to *F. arabica* with 35%. Four species were grouped in the second subcluster. *F.glutinosa* and *F. microphylla* were closly related with similarity 80% and hadhomology percentages 52 and 42% with *F. sinaica* and *F. indica*, respectively. The second cluster was related to the first one with homogeniety 20% and included only *F. schweinfurthii.* The genetic relationship between the third and the second clusters was 19% and, the third one cosisted of *F. tenuifolia* and *F. thebaica* with similarity 34%.

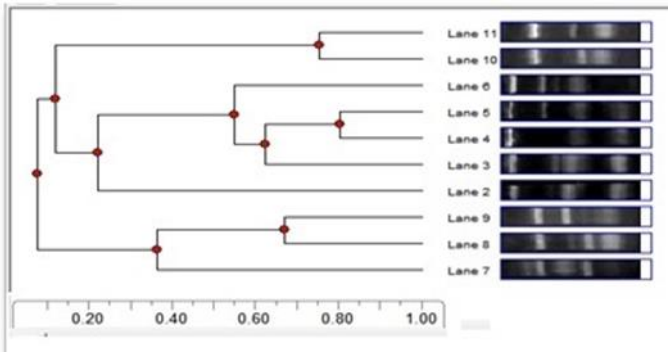
For primer-5, two clusters were indicated with homology percentage 5%. The first one diveded into two subclusters with similarity 9%. The first subcluster grouped *Fagonia indica* and *Fagonia sinaica* with homogeneity 34% and, both species were similar to *Fagonia cretica* with 22%. The second subcluster included *Fagonia schweinfurthii* and *Fagonia tenuifolia* with homology percentage 21%. The second cluster divided also into two subclusters with similarity 15%. *F.arabica* and *F. glutinosa* grouped in the first subcluster with homogeniety 33% and, both species were related to *F.bruguieri* with homology percentage 26%. The second subcluster consisted of *F.microphylla* and *F. thebaica* with 26% similarty.

Primer-6 exhibited two main clusters with homogeneity < 5%. The first one grouped *F.bruguieri* and *F. indica* with genetic homology 40% and, both species were similar to *F. sinaica* with 25%. The second cluster divided into two subclusters with homology percentage 5% and, the first subcluster contained only *F. schweinfurthii.* The second one subdivided into two sub-subcluster. The first one included only *F. thebaica* with similarity 26% and, five species were grouped in the second sub-subcluster. The most related species were *F. arabica* and *F. microphylla* with homology percntage 53%. *F. cretica* and *F. glutinosa* were also similar with 45%. The four species were related to *F.thebaica* with 31% similarity.

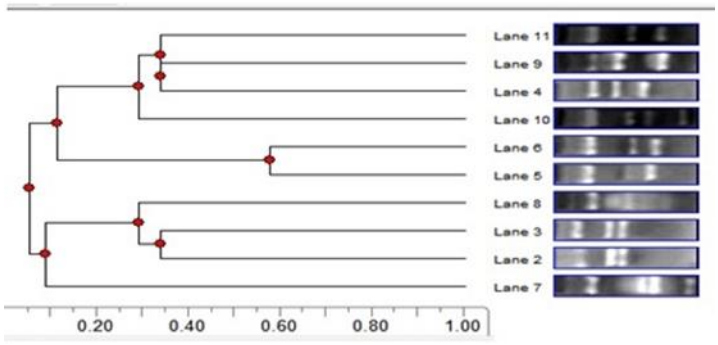
Primer-1



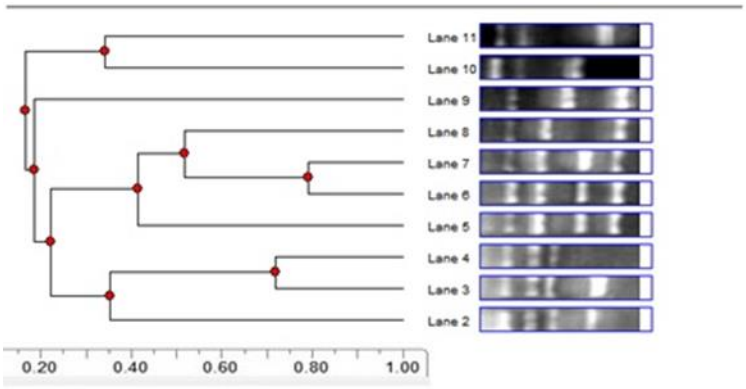
Primer-2



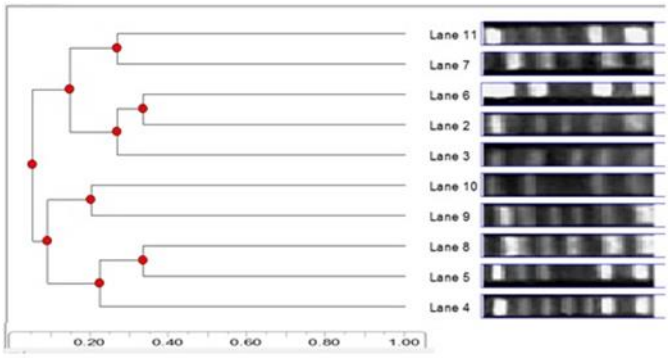
Primer-3



Primer-4



Primer-5



Primer-6

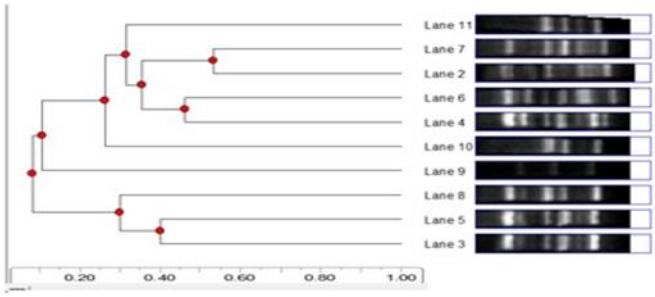


Fig.3. Dendrograms presenting the phyllogenetic relationships among the ten *Fagonia* species based on each primer data. Lanes 2-11: *Fagonia arabica; Fagonia bruguieri; Fagonia cretica; Fagonia indica; Fagonia glutinosa; Fagonia microphylla; Fagonia sinaica; Fagonia schweinfurthii; Fagonia tenuifolia and Fagonia thebaica*, respectively.

Figure (4) presents the dendrogram of all primers via UPGMA method according to RAPD fingerprinting. Two clusters with homology percentage 9% were indicated. The first one contained *F. microphylla* and *F. schweinfurthii* with similarity 50%. The second cluster divided into two subclusters. The first one included three *Fagonia* species (*F. cretica*, *F. indica* and *F. glutinosa*). The homogeniety between *Fagonia cretica* and *F. indica* was 50% and, both species related to *F. glutinosa* with 40%. The second subcluster subdivided into two sub-subclusters. The first one contained *F.arabica* and *F. bruguieri* with 50% similarity. The other sub-subcluster

grouped *F. sinaica* and *F. thebaica* with homogeniety 50% and, both species in genetic relatioship 40% with *F. tenuifolia.*

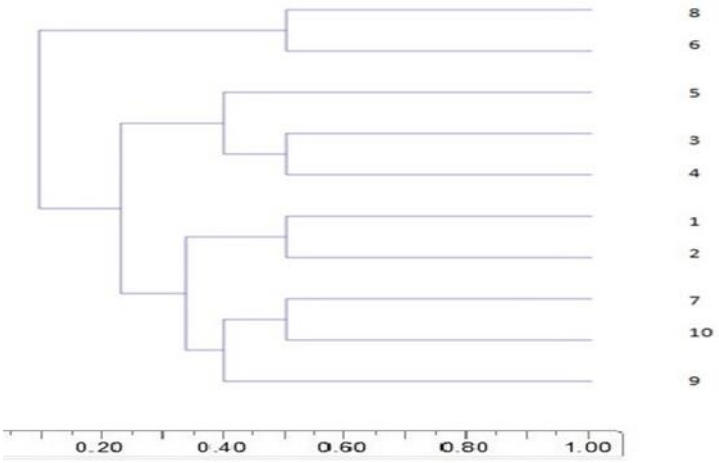


Fig. 4. Dendrogram showing the phyllogenetic relationships among the ten *Fagonia* species based on all primers data. 1-10: *F. arabica, F. bruguieri, F. cretica; F. indica, F. glutinosa, F. microphylla, F. sinaica, F. schweinfurthii, F. tenuifolia and F.thebaica*, respectively.

4. DISCUSSION

Genus *Fagonia* belongs to subfamily Zygophylloideae which is the largest subfamily of the Zygophyllaceae and consists of six genera; *Zygophyllum, Fagonia, Augea, Roepera, Tetraena* and *Melocarpum* (Beier et al., 2003, Bellstedt et al., 2008). Genus *Fagonia* considered as one of the most difficult genera in the circumscription of the species belonging to it. This genus is objected to many taxonomic investigations to clarify the most significant relations between its species. From the most important taxonomical studies are those of Quezel (1956), El-Hadidi (1966), Batanony and Batonony (1970) and Taia (2015, 2016, 2017 & 2021). The morphological, floral and anatomical investigations done by (Taia *et al*., 2015, 2016 & 2017) clarified that the vegetative characters can help in identifying some species. They clarified that the spiny stipules beside the leaf micro-morphological characters can be of help in the identification of some species. From the most noticeable character which can be important taxonomic one in grouping of the species is the presence of the knee-like structure in the style of the carpels in some species, this add new characters in the delimitation of the taxa (Taia et al., 2016). Internal structures especially the pith shape can be of use in the grouping of the species as mentioned before by Boissier (1867). El-Hadid (1966) and Taia et al. (2017) found that the internal structure variations can help in the discrimination of the species. Palynological studies on this genus are few and did not give valuable opinions about the delimitation of the species. For that, this investigation has been done as a trial to clarify the Molecular variations among the Libyan species.

Molecular analyses of the *Fagonia* species have not investigated till now and previous works dealt with the origin and distribution of some species. Accordingly, this work considered from the first

works in this concern. The data obtained from the DNA analysis using primer 1 indicated that *F. indica* and *F.glutinosa* are very closely related as well as *F.cretica*; *F.microphylla* and *F.arabica*, which came together in another group. These groupings don't coincide with the clustering of the species according to their vegetative morphological ones (Taia et al., 2015), but partly in agreement with the floral morphological characters (Taia et al., 2016). The genetic similarity between both *F.sinaica* and *F.schweinfurthii* cannot be noticed externally by their vegetative morphological characters, but the genetic similarity between *F.tenuifolia* and *F.thebaica* is obvious morphologically by both vegetative and floral characters. The same results obtained by using primer 2, as both *F.cretica* and *F.indica* are morphologically and anatomically different, while *F.bruguieri* and *F.glutinosa* partly share their floral characters.

Primer 3, grouped the studied taxa in two main groups, all of them in one homogenous group while *F.microphylla* alone in another group. This result indicates that all the *Fagonia* species are closely related and coincide with the other morphological and anatomical investigations. Primers 4 and 5 grouped the taxa into two main groups and kept both *F.sinaica* and *F.indica* as well as *F.tenuifolia* and *F.thebaica*, each two, closely related. Primer 6 bring *F.sinaica* and *F.indica* together, as the previous primers, but *F.thebaica* and *F. schweinfurthii*, each came in a separate subgroups, while *F.cretica* and *F.glutinosa* came together and both closely related to *F.thebaica*.

The dendrogram of all primers via UPGMA method according to RAPD fingerprinting gave two clusters with homology percentage 9%. The first one containing *F.microphylla* and *F.schweinfurthii* with similarity 50%. The second cluster divided into two subclusters. The first one included three *Fagonia* species (*F.cretica; F.indica* and *F.glutinosa*). The second subcluster subdivided into two sub-subclusters. The first one contained *F.arabica* and *F.bruguieri* with 50% similarity. The other sub-subcluster grouped *F.sinaica* and *F.thebaica* and, both species in genetic relationship with *F.tenuifolia*. Meanwhile the division of the studied taxa is not the same in each tool separately. This reveals that the delimitation of the *Fagonia* species is still unclear and the relation between the species is confusing as well. For better understanding the genetic relationship between the *Fagonia* species more molecular and chemical analyses needed for better taxonomic classification and genetic relations.

From this study we can conclude that the Molecular analysis done of genus *Fagonia* are of limited help in the classification of the genus, and more detailed analyses needed. Also the taxa within that genus need further breeding experiments to investigate the delimitations within its taxa.

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