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# Biodiversity of some fig cultivars in Southern Syria

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## ABSTRACT

Genetic diversity of fourteen cultivars of figs (*Ficus carica* L.) growing in Swaida governorate which is located in the south region of Syria were investigated using (Simple Sequence Repeats) SSRs technique. The four cultivars were collected from local fields without any scientific taxonomy and the farmers did not classify them properly. Eight pairs of SSRs markers were used depending on their ability to separate between fig cultivars used in previous studies, six pairs of them gave amplified products while MFC3 and MFC6 primers did not give any amplification. A total of 17 alleles were detected at six SSRs loci. The alleles number per locus ranged from 2 to 4 with an average of 2.83 alleles/locus. The observed heterozygosity (Ho) was 0.33, while the expected heterozygosity (He) was 0.17. The mean value of genetic similarity was 0.69 where fig cultivars have separated into two clusters in Cluster Analysis, which confirms a significant genetic similarity between most of the cultivars. MFC1 and MFC2 loci gave about 0.67 and 0.61 PIC (Polymorphism Information Content) values respectively, which confirm their ability to study the genetic diversity of fig cultivars more than other loci. F1 and F2 cultivars greatly affect the quality of the fruits as paternity traits as Caprifigs. Tammozi cultivar has low values of its genetic similarity with the rest of the cultivars the Generally, the challenges in this study were in characterizing unrecognized fig cultivars in southern Syria to distinguish between them as they were not certified in the agriculture ministry in Syria of the present study able the SSR markers differentiated the fig cultivars in Syria.

**Keywords:** Simple Sequence Repeats (SSRs), Molecular Classification, Figs, PCR.

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## INTRODUCTION

The Mediterranean basin is a natural habitat for genetic resources of global importance (Dowdeswell, 1995). Fig (*Ficus carica* L.) is a useful genetic resource for commercial cultivation (Chatti *et al.*, 2010) and the fig tree is one of the oldest fruit trees known to humans in a long time (Aksoy, 1998). Syria is the sixth world producer of figs with an estimated production of 38,035,000 tons and a cultivated area of 9,422 hectares in 2018, where Idlib governorate ranked first according to the cultivated area, number of trees, and productivity, while in Swaida governorate, the total cultivated area was 320 hectares with a production of 1,779 tons (Annual Agricultural Statistical Group, 2018). Figs (*Ficus carica* = 26 chromosomes) belong to the Moraceae family, which contains approximately 1,400 species distributed within 40 genera. Ficus genus comprises approximately 750 species divided into six races, which share a unique flowering style, where flowers are carried in a fruit called the syconium (Berg, 2003). The long history of domestication and cultivation with widespread dispersal of cultivars with many synonyms has resulted in a great deal of confusion in the identification and classification of cultivars in fig. (Aradhya

*et al.*, 2010). The extensive and indiscriminate exploitation of these genetic resources led to a deterioration in natural resources and a severe shortage of biodiversity in the region, which necessitated the preservation, characterization, classification, and multiplication of these resources to use them in future breeding programs for Syrian fig cultivars. This work aimed to study the genetic diversity of fourteen cultivars of figs (*Ficus carica* L.) growing in Swaida governorate in southern Syria using SSRs (Simple Sequence Repeats) technique. The importance of the varieties used lies in being the most productive qualitatively and quantitatively, as many unnamed numerous genotypes are present in the region likewise other regional studies in turkey (Akin *et al.*, 2020). Hence, it is imperative to pay particular attention to endangered and rare cultivars (Debbabi *et al.*, 2021) for characterization.

## MATERIALS AND METHODS

This study was carried out in the Department of Horticultural Sciences and the Laboratories of Biotechnology at the Faculty of Agriculture, Damascus University, in 2019 and 2020.

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## Plant material

Ten local cultivars of figs (Figures 1-14) obtained from the gene bank collection of the General Commission of Scientific Agricultural Research (site Haut), and four wild-growing cultivars in the Areeka site were investigated in this study (Table 1)

## Molecular study

Fresh leaves of fig 3 to 4-week-old growths, free of pathological and insect injuries, were collected at the end of April from one representative tree of each cultivar. The samples were washed with distilled water, placed in transparent plastic bags, and stored at -80 °C until use.

## DNA extraction

DNA was extracted from leaves using the cetyltrimethylammonium bromide (CTAB) method described by Chatti *et al.* (2010). The quantity of the extracted gDNA was determined by measuring the UV absorbance at 260 and 280 nm using a spectrophotometer. The quality of extracted DNA was determined by gel electrophoresis on ethidium bromide-stained 1% agarose. DNA was then used as a template for subsequent amplification using PCR.

## Simple Sequence Repeats (SSRs)

### DNA Amplification

Eight pairs of SSRs markers were selected because of their high polymorphism in the fig family (Khadari *et al.*, 2001). The appropriate annealing temperature for each pair of primers was calculated, where temperatures varied by the number of nucleotides and the GC% (Table 2, Khadari *et al.*, 2001). The PCR reaction was performed in 25 µl final volumes with a mixture of 50 ng of genomic DNA, 1X Taq buffer, 0.32 µM dNTP, 1.5 mM MgCl<sub>2</sub>, 4 µM of each forward and reverse primers and 0.2 U/µl Taq DNA polymerase supplemented with water to 25 µl. The PCR conditions were as follows: pre-denaturation at 95 °C for 5 min; denaturation at 95 °C for 1 min, annealing

**Table 1: Codes and Names of fig cultivars investigated in this study**

Code	Name
1	F (4) Areeka
2	F (1) Areeka
3	F (2) Areeka
4	F (3) Areeka
5	Small Bokrati
6	Bokrati
7	Moloki
8	Sokari
9	Dwashi
10	Bechari
11	Karima
12	Bragi
13	Swaidi
14	Tammozi



**Figure 1: F2 cultivar fruit and leaf shape**



**Figure 2: Karima fruit and leaf shape**



**Figure 3: Bechari fruit and leaf shape**



**Figure 4: Moloki fruit and leaf shape**



**Figure 5: F3 cultivar fruit and leaf shape**



Figure 6: F1 cultivar fruit and leaf shape



Figure 11: Tammozi cultivar fruit and leaf shape



Figure 7: Bragi cultivar fruit and leaf shape



Figure 12: Bokrati cultivar fruit shape



Figure 8: Swaidi cultivar fruit and leaf shape

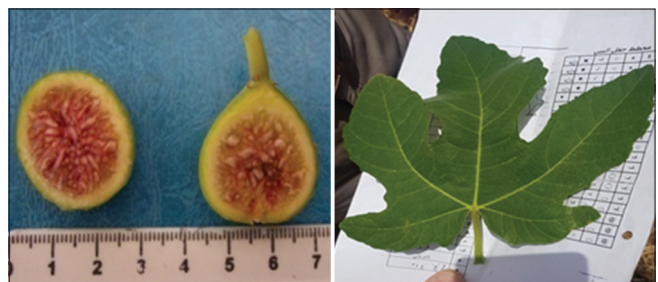


Figure 13: Small Bokrati cultivar fruit and leaf shape



Figure 9: Sokari cultivar fruit and leaf shape



Figure 14: F4 cultivar fruit and leaf shape

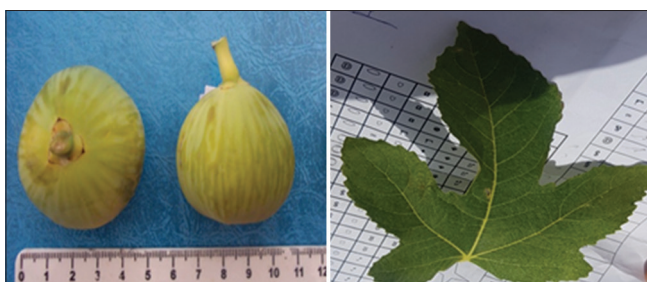


Figure 10: Dwashi cultivar fruit and leaf shape

at an appropriate temperature for each primer for 1 min, and extension at 72 °C for 1 min and final extension at 72 °C for

10 min. Amplified products were analyzed using 3% agarose gel electrophoresis, and visualized under a UV transilluminator.

### Statistical analysis

The degree of genetic similarity was determined by calculating the convergence ratio matrix and drawing the genetic similarity tree. Any presented allele was encoded by number 1, while alleles that did not present were encoded by 0. The molecular weights of DNA bands were read using the gel Quant program (ver 1.8.2) (Lazar, 2010). Then, using the convergence ratio matrix (Dice, 1945) by Paleontological Statistics program (ver. 1.67), the genetic

**Table 2.** The nucleotide sequence of SSR primers GC % and the number of repeats per primer (frequency) with the appropriate annealing temperature for each pair

Primer	Band Size	The nucleotide sequence	GC%	frequency	Annealing temperature (c)
MFC1	192	ACTAGACTGAAAAACATTGC TGAGATTGAAAGGAAACGAG	36.5	[CT] <sub>13</sub>	55
MFC2	172	GCTTCCGATGCTGCTCTTA TCGGAGACTTTTGTCAAT	44.7	[AC] <sub>18</sub> [AT] <sub>7</sub>	55
MFC3	136	GATATTTTCATGTTTGTGTTG GAGGATAGACCAACAACAAC	34.1	[AC] <sub>15</sub> T[AC] <sub>8</sub> [AT] <sub>7</sub>	55
MFC4	218	CCAAACTTTTAGATACAACCTT TTTCTCAACATATTAACAGG	29.2	[AT] <sub>4</sub> [AC] <sub>11</sub>	55
MFC5	140	ACCAATCCAATAATAATCC ACACGCTTACTAGAATTACC	35	[GA] <sub>13</sub>	50
MFC6	313	AGGCTACTTCAGTGCTACA GCCATAAGTAATAAAAACC	39.4	[TAA] <sub>3</sub> [GT] <sub>8</sub>	50
MFC7	150	CACAATCAAATAGTTACCG AGCGAAGACAGTTACAAAGC	39	[AG] <sub>11</sub>	50
MFC8	179	GTGGCGTCGCTCTAATAAT TATTCTATGCTGCTTATGTCA	38	[CA] <sub>9</sub> T[CA] <sub>14</sub> [TA] <sub>6</sub>	50

similarity tree between cultivars was drawn using the PAST statistical program (Hammer *et al.*, 2001). The total number of alleles generated in each locus and the PIC (Polymorphic Information Content) were calculated according to Botstein (Botstein *et al.*, 1980), also Expected heterozygosity (He) and observed heterozygosity (Ho) was calculated according to:  $He = 1 - \sum Pi^2$  (Powell *et al.*, 1996), Where Pi is the frequency of the allele in the I locus. Ho is the heterogeneous proportion of individuals, calculated using the Gene Alex program (Peakall & Smouse, 2012).

## RESULTS AND DISCUSSION

### Polymorphism among fig cultivars

SSR polymorphism results from the difference in the number of repetitive units in the loci of simple sequence repeat when using pairs of primers designed according to the area around the locus, thus it is determined by the amplification products lengths in the polymerase chain reaction PCR. Six of the eight pairs of primers used on the fig cultivars gave amplified products in the polymerase chain reaction, while MFC3 and MFC6 primers did not give any amplification.

A total of 17 alleles were detected at six SSRs loci in this study while in another (Sadder *et al.*, 2021) study a total of 124 alleles were detected with 310 accessions and 14 SSR loci. The allele number per locus ranged from 2 to 4. In other studies, 3.59 alleles per locus were detected (Boudchicha *et al.*, 2018). Knap *et al.* (2001) showed that the detected allele number per locus ranged from 2 to 5. In contrast, the allele number per locus was higher in other studies, where it was ranged from 3 to 6 (Millar & Westfall, 1992) and from 4 to 9 alleles per locus with 194 breeds due to the greater number of SSR loci (Saddoud *et al.*, 2007). The average number of alleles in our study was lower than that showed by Giraldo *et al.* (2005) with 3 alleles/locus. Ahmed *et al.* (2015) used 17 SSR loci to characterize 71 cultivated and wild Tunisian fig-trees revealing a total of 74 alleles. 91 alleles were detected with an average of 13 alleles per locus (Ganopoulos *et al.*, 2015). 12 local cultivars and

two caprifigs, in addition to 15 reference samples have been fingerprinted using 21 SSR markers, a total of 77 alleles were detected (Perez-Jiménez *et al.*, 2012).

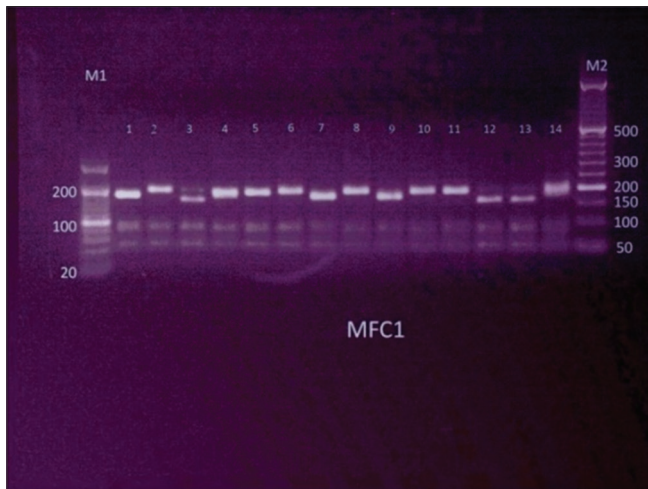
This relative decrease in the average number of alleles in our study compared to the previous studies may be due to the same origins of cultivars, and to the few numbers of strains and primers used. The difference in the effectiveness of each locus is likely to be due to the number of cultivars, which reflects a greater chance to find more alleles as the number of polymorphic patterns increases. A total of 20 polymorphic patterns between the cultivars were identified, with MFC1 and MFC2 giving the highest number of polymorphic patterns (5 patterns) (Figures 15 and 16), followed by MFC7 (Figure 17), and the rest of the primers gave only two patterns of polymorphism (Figures 18 and 19) with an average of 3.17 (Table 3). The number of patterns that appeared in our study was lower than those patterns that appeared in Khadari *et al.* (2001) and Saddoud, 2007) studies, and that is due to the high number of samples studied in their studies in comparison to our study. The difference in the number of alleles per locus is explained by the structure of the repetitive units in it, so the variation is lower when the repetitive units in the locus are simple and short.

1-F4, 2-F1, 3-F2, 4-F3, 5- Small Bokrati, 6- Bokrati,7- Moloki, 8- Sokari, 9- Dwashi, 10-Bechari, 11- Karima, 12- Bragi, 13-Swaidi, 14- Tammozi

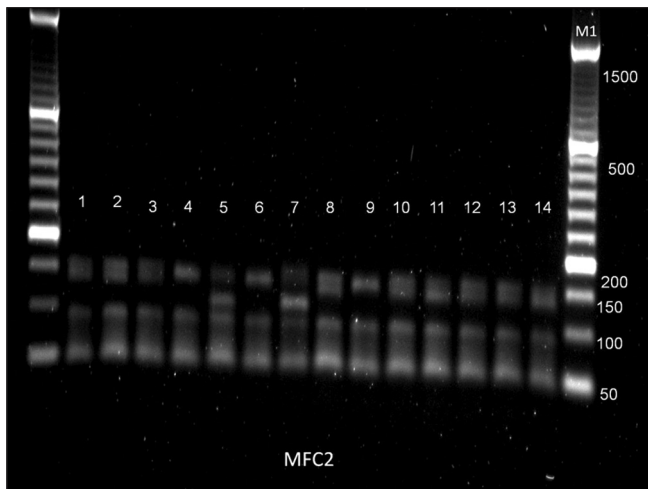
1-F4, 2-F1, 3-F2, 4-F3, 5- Small Bokrati, 6- Bokrati,7- Moloki, 8- Sokari, 9- Dwashi, 10-Bechari, 11- Karima, 12- Bragi, 13-Swaidi, 14- Tammozi

M1 Standard marker 50 bp. 1-F4, 2-F1, 3-F2, 4-F3, 5- Small Bokrati, 6- Bokrati,7- Moloki, 8- Sokari, 9- Dwashi, 10-Bechari, 11- Karima, 12- Bragi, 13-Swaidi, 14- Tammozi

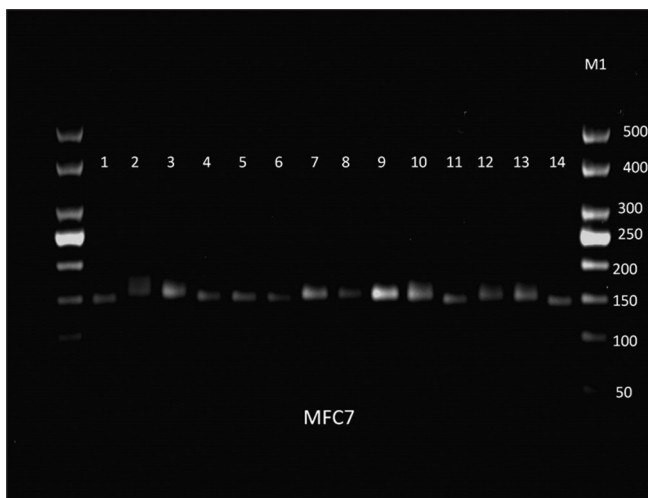
1-F4, 2-F1, 3-F2, 4-F3, 5- Small Bokrati, 6- Bokrati,7- Moloki, 8- Sokari, 9- Dwashi, 10-Bechari, 11- Karima, 12- Bragi, 13-Swaidi, 14- Tammozi



**Figure 15:** DNA bands resulting from MFC1 amplification where the numbers 1-14 indicate fig cultivars codes, M1 Standard marker 20 bp, M2 Standard marker 50 bp



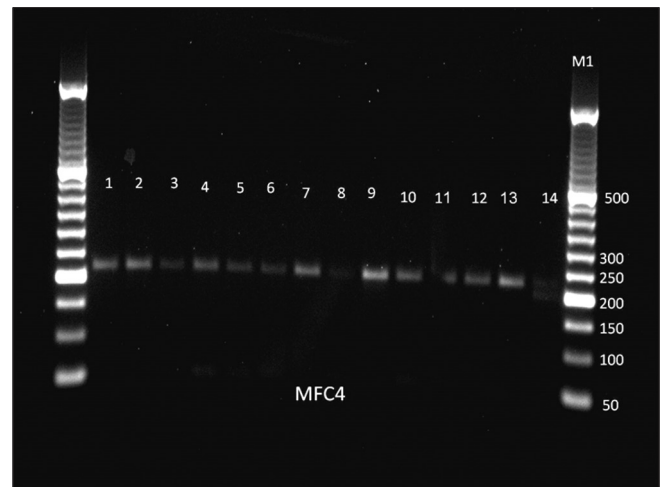
**Figure 16:** DNA bands resulting from MFC2 amplification where the numbers 1-14 indicate fig cultivars codes. M1 Standard marker 50 bp



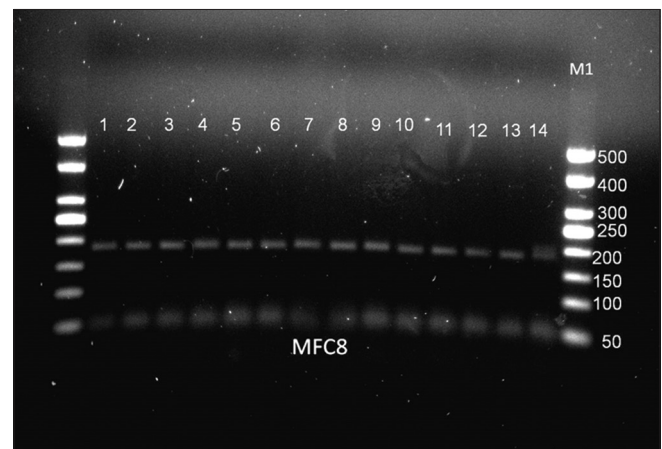
**Figure 17:** DNA bands resulting from MFC7 amplification where the numbers 1-14 indicate fig cultivars codes

In all the studied cultivars, the observed heterozygosity (mean  $H_o = 0.33$ ) was higher than the expected heterozygosity (mean  $H_e = 0.17$ ). In other studies, the expected heterozygosity ranged from 0.071 to 0.796 and the observed heterozygosity ranged between 0.071 and 1.0000 (Ali, 2019), while  $H_o$  ranged from 0.18 to 0.76 and  $H_e$  ranged between 0.24 and 0.81 (Eoman *et al.*, 2017) meanwhile in (Sadder *et al.*, 2021) study  $H_e$  and  $H_o$  values were 0.652 and 0.685, respectively.

Six primers (75%) of the total primers used were polymorphic. Polymorphism was lower in MFC4 and MFC8 (about 0.13), and that is maybe because the cultivars are from the same region. To determine the best loci for the cultivars characterization, primers were classified according to the number of polymorphic patterns, MFC1 and MFC2 gave the highest number of patterns 5, while MFC7 gave 3 patterns and 3 alleles, the remaining loci resulted in 2 alleles and 2 patterns. The band's molecular weights are directly related to the primers' structures (Huang *et al.*, 2002), so the calculation of PIC for each primer is an essential criterion indicating the ability of the primer to distinguish and show genetic



**Figure 18:** DNA bands resulting from MFC4 amplification where the numbers 1-14 indicate fig cultivars codes. M1 Standard marker 50 bp



**Figure 19:** Unique band resulting from MFC8 primer in Tammozi cultivar, M standard marker 50bp

variations between different cultivars, thus the closer the PIC value is to 1, the greater the ability to distinguish and show genetic variations. In our work, the values of PIC were low to medium, ranging from 0.13 to 0.67, (Table 3) while in other studies PIC ranged from 0.67 to 0.85 (Saddoud *et al.*, 2011), which indicates that the loci of SSR in our study present a low to medium ability to distinguish the cultivars and show the genetic variation between them because the cultivars have the same origins, which in turn increases their homozygous characteristics. Based on the resulting PIC values, MFC1 can be distinguished with the highest value of PIC 0.67 followed by MFC7 with a PIC value of 0.64. Mafrica *et al.* (2015) showed that the mean PIC was 0.51. The number of alleles detected in each cultivar ranged between 6 to 9, with the highest number in moloki and Tammozi cultivars

### Distinguish fig cultivars using unique bands

Some of the SSR markers were able to distinguish unique bands in Tammozi cultivar such as (Figure 5) which has unique band in MFC8 primer with 205 bp molecular weight and another unique band appeared in MFC1 primer with 200 bp.

1-F4, 2-F1, 3-F2, 4-F3, 5- Small Bokrati, 6- Bokrati, 7- Moloki, 8- Sokari, 9- Dwashi, 10-Bechari, 11- Karima, 12- Bragi, 13-Swaidi, 14- Tammozi

### Genetic similarity between cultivars

The degree of genetic similarity between cultivars was determined by the Dice coefficient (Table 4), where the

highest degree of genetic similarity was between Bokrati, and F3 (1.00), and Bragi and Swaidi (1.00), while the lowest genetic similarity was between Tammozi and F3, and between small Bokrati, Bokrati, and Dwashi (0.375). The mean value of genetic similarity was 0.69. Akin *et al.* (2020) showed genetic similarity between 0.24 and 0.90 which confirms a significant genetic convergence between most of the cultivars in our study, except Tammozi cultivar which revealed low similarity with the rest of the cultivars with an average similarity of 0.57, lower than the general average of similarity and that is maybe due to the difference of origin. The results could not distinguish between Bokrati and F3 or between Bragi and Swaidi in any of the investigated loci, and that is maybe due to the high morphological similarity between them or because they differ by only one or few numbers of simple sequence repeats (Di Gaspero *et al.*, 2000).

### UPGMA Cluster analysis of fig cultivars

Cluster analysis divided cultivars into groups, reflecting the degree of genetic similarity among them. Generally, cultivars may be a group in a single group according to their geographical origin, or gender (Hormaza, 2002). The cultivars in this study were separated into two main groups (Figure 20) at a similarity level of 0.69, and this distribution is supported by a 100% of reliability. In the second group B, F3 and Bokrati are incomplete symmetry at the level of 83% of reliability, and small Bokrati was associated with them at a 0.87 of similarity, Dwashi linked to b1 subgroup at a 29% of reliability, and these results are agreement with previous studies in Kurdistan Region-Iraq

Table 3. Number of alleles resulting from primer pairs used in SSR (Na), Polymorphic patterns, polymorphic information content (PIC), and bands lengths (bp) for each primer pair

Primer name	Band lengths (bp)	polymorphic information content (PIC)	Polymorphic patterns	Number of alleles (Na)
MFC1	170-200	0.67	5	4
MFC2	130-170	0.61	5	4
MFC4	200-230	0.13	2	2
MFC5	130-150	0.49	2	2
MFC7	150-170	0.64	3	3
MFC8	200-205	0.13	2	2
Mean	175	0.45	3.17	2.83

Table 4. Genetic similarity degree between fig cultivars based on dice coefficient

Cultivar	F4	F1	F2	F3	Small Bokrati	Bokrati	Moloki	sokari	dwashi	Bechari	karima	Bragi	Swaidi	Tammozi
F4	1													
F1	0.750	1												
F2	0.625	0.875	1											
F3	0.533	0.667	0.533	1										
Small Bokrati	0.400	0.533	0.533	0.857	1									
Bokrati						1								
Moloki	0.706	0.706	0.588	0.625	0.625	0.625	1							
Sokari	0.571	0.714	0.714	0.769	0.769	0.769	0.400	1						
Dwashi	0.667	0.533	0.400	0.857	0.714	0.857	0.750	0.615	1					
Bechari	0.667	0.800	0.800	0.714	0.714	0.714	0.500	0.923	0.571	1				
Karima	0.800	0.800	0.800	0.571	0.571	0.571	0.500	0.769	0.429	0.857	1			
Bragi	0.667	0.800	0.933	0.429	0.429	0.429	0.625	0.615	0.429	0.714	0.714	1		
Swaidi	0.667	0.800	0.933	0.429	0.429	0.429	0.625	0.615	0.429	0.714	0.714	1	1	
Tammozi	0.706	0.588	0.588	0.375	0.375	0.375	0.444	0.533	0.375	0.625	0.750	0.625	0.625	1

(Hussien *et al.*, 2020). Group A was divided into three subgroups, where Tammozi came associated in the highest hierarchical level with a similarity of 0.61. The first subgroup a1 included F4 and moloki together at a similarity of 0.71, while the second subgroup a2 match Bragi and Swaidi at 56% of reliability, associated with F2 at 92% of similarity. F1 also came with them at 0.82 of similarity. The third subgroup a3 included Sokari and Bechari with Karima at 0.82 of similarity. In comparison with another study, UPGMA cluster analysis grouped caprifig accessions in three groups (Essid *et al.*, 2015). High-reliability values within subgroups (> 0.60%) confirm that cultivars which came together are genetically similar. For example, the presence of Bragi and Swaidi at a similarity level of 1.00 match with F2 at a 56% of reliability, indicating their involvement in the same loci with minor differences.

According to the genetic similarity tree, groups of cultivars are associated with each other at the molecular level, as well as they can be described as very similar in their morphological characteristics. The high similarity of these cultivars indicates that it is necessary to analyze them using a greater number of SSR loci that are directly associated with genes responsible for quantity or quality characteristics, to separate each of them

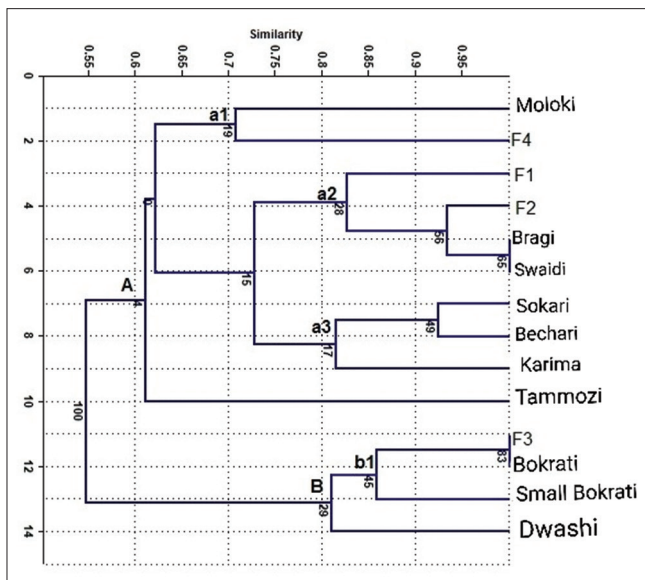


Figure 20: Cluster analysis of fig cultivars based on Dice coefficient

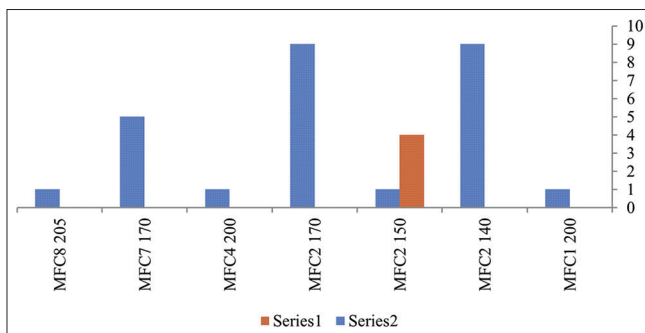


Figure 21: The distribution of alleles in fig cultivars, series1 is group B, series2 is group A

more accurately and clearly. The high symmetry between Small Bokrati, Bokrati as well as F3 because they are likely to be a single cultivar. The distribution of cultivars in the similarity tree (Figure 21) was mainly related to the distribution of alleles. All fig cultivars which contain alleles 140, 170 bp at MFC2 locus were placed in the first group, as well as all cultivars which contain alleles 150 bp at MFC2 locus were placed in the second group.

## CONCLUSION

We have investigated 14 fig cultivars in southern Syria to determine the genetic relationship between them in order to identify their genetic identities and certify them in the ministry of agriculture as commercial cultivars. The conservation of genetic resources of figs, multiplication and breeding of masculine figs (caprifigs) such as F1 and F2 that greatly affect the quality of the fruits as paternity traits. The greater number of primers used in the study which helps for identification of the unique SSR alleles for characterization of fig trees in nurseries, and fields for purity assessment.

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