Original scientific paper
DOI: /10.5513/JCEA01/24.3.3933
Journal of Central European Agriculture, 2023, 24(3), p.736-748

Evaluation of genetic diversity in two *Morus* species in Syria, using molecular markers ISSR and SSR

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Received: April 13, 2023; accepted: July 30, 2023

ABSTRACT

In Syria, there are ancient wild, and cultivated mulberry trees, traditionally used to feed the silkworm *Bombyx mori* L., and cultivated for their edible fruits. Evaluating genetic relationships is imperative for the identification, conservation, and improvement of mulberry germplasm. The present study addresses the use of inter simple sequence repeat (ISSR) and simple sequence repeat (SSR) markers to estimate genetic diversity within and between two different mulberry species (49 genotypes of *Morus nigra* cultivar. El-Shami) and (8 genotypes of *Morus alba*) from different regions of Syria (Al-Qunaitra, Tartus and Latakia). Fifteen ISSR primers and six SSR primer pairs were used in the analysis. Overall, genetic diversity within *M. alba* and *M. nigra* was low (0.22). The genetic distance between the two species (*M. nigra* and *M. alba*) was higher (0.72) than that within the species (0.3). A dendrogram based on SSR and ISSR results showed the clustering of samples according to the species and to their geographic collection sites. The level of polymorphism detected between samples using the ISSR marker (90.31%) was higher than that detected with SSR marker (68.05%). The genotypes collected from Al-Qunaitra were very similar as the coefficient of genetic distance -CGD- was less than 0.1, while genotypes from Latakia were more distant (CGD is 0.33). In general, the level of genetic diversity between the analyzed genotypes of *M. nigra* cv. El-Shami was lower than between the samples of *M. alba*.

Keywords: Genetic similarity, *Morus nigra*, *Morus alba*, DNA polymorphism

INTRODUCTION

The genus *Morus* belongs to the Moraceae family and grows wild and cultivated. Taxonomic studies have shown great genetic variability and adaptability of the genus species to different agro-climatic conditions. Mulberries are deciduous trees or shrubs, cross-pollinating and highly hybridized. Their leaves are used as the exclusive food for the domesticated silkworm, *Bombyx mori*. A few species of mulberry (*M. alba*, *M. nigra*, and *M. rubra*) are used for their edible and healthy fruits. Despite the fact, that the conservation of the genetic heritage of mulberry trees has been studied in particular for sericulture, the interest in mulberries as healthy fruits has expanded considerably (Atmakuri et al., 2009).

White mulberry includes predominantly diploid cultivars with 28 chromosomes, but may also occasionally exist in the triploid state. Black mulberry (*Morus nigra*) has a very high degree of ploidy (2n = 22x = 308 chromosomes) (Honnegowda Venkatesh, 2021; Yamanouchi et al., 2017).

The characterization and conservation of mulberry germplasm are helpful for saving existing genetic resources. As part of tradition, the genetic diversity of the genus *Morus* has been evaluated using external morphology and physiological traits (Katsumata, 1972), but these taxonomy traits are still ineffective in estimating the diversity and relationships among different *Morus* species, due to environmental influences, as well as to the fact that mulberry is a heterogeneous tree and can

be easily hybridized and propagated in both asexually and sexually ways. These features make the genetic background of *Morus* more complex (Dandin, 1998). Recently, some alternative techniques based on molecular markers, such as AFLP (Sharma et al., 2000; Kafkas et al., 2008), RAPD (Orhan et al., 2007; Iruela et al., 2002), ISSR (Vijayan and Chatterjee, 2003; Vijayan, 2003; Vijayan et al., 2004a,b; Arvind et al., 2004; Zhao et al., 2006; Zhang et al., 2011; Ju-Eun et al. 2020; Kumara et al., 2022), and SSRs (Aggarwal and Udaykumar, 2004; Zhao et al., 2005; Wani et al., 2013), have been used to evaluate the genetic diversity within and between mulberry species and varieties, which are essential for estimating and understanding the relationships among *Morus* genotypes. It was noted in many studies that genetic variations between species were higher than that within species (Abed-Elkader, 2014; Un-Hyang et al., 2021).

Inter simple sequence repeats (ISSRs)- PCR technique is a simple method, based on the use of microsatellite sequences as a single primer in a polymerase chain reaction to amplify DNA sequences and generate multi-locus markers. The ISSR is a dominant molecular marker able to detect high levels of DNA polymorphism over the genome. (Nagaoka and Ogihara, 1997).

Microsatellites or simple sequence repeats (SSRs)-PCR techniques are based on the use of primer pairs designed from conserved DNA sequences to amplify Microsatellite DNA motifs. They are locus-specific markers and highly polymorphic. They have been shown to be efficient in genetic analyses of mulberry, due to their multi-allelic nature and co-dominant segregation patterns (Zhang et al., 2006; Tan et al., 2014). The application of SSR markers requires a previous characterization of a set of microsatellite loci of the studied species' genome. Aggarwal et al. (2004) presented the first report on the isolation of six microsatellite markers of mulberry, developed from *Morus indica*, and a broad cross-species affinity (*M. nigra*, *M. alba*, *M. bombycis*) suggesting the wider potential of the new markers in genetic analysis of *Morus* resources. SSRs have been assayed on *M. nigra*, *M. rubra* and *M. alba* genotypes in Turkey, the outcomes suggest that *M. alba* genotypes had a great diversity than the red and black mulberry genotypes (Orhan et al., 2020).

The molecular characterization and analysis of *Morus alba* L. cultivars from Cuba, Costa Rica, Brazil, South Korea, Ethiopia, China, Japan, Italy and Spain with SSR markers revealed a genetic distance of 0.570 among the genotypes tested (Garcia-gómez et al., 2019). ISSR and SSR markers have been applied to study mulberry variation and relationships amongst cultivated and wild genotypes (Zhao et al., 2007), and also the use of the same markers showed a high level of genetic diversity within mulberry trees in Lebanon (Kadri et al., 2021).

In Syria, there were few studies on the assessment of mulberry genetic diversity. For instance Abed-Elkader (2014) studied the genetic diversity of mulberry in the northwestern region of Syria using AFLP analysis. There was a lower level of genetic diversity between *M. nigra* genotypes compared to *M. alba* genotypes. The objective of the present study was to focus on the evaluation of genetic variations within and between two cultivated mulberry species, *M. nigra* cv. El-Shami, and *M. alba* through ISSR and SSR markers, in two different regions of Syria.

MATERIALS AND METHODS

Plant materials

Young leaf samples were collected from 57 mulberry trees. 49 of them were cultivated monoecious *M. nigra* (El-Shami) trees, including 35 trees from a field in Al-Qunaitra governorate and two trees within another field (Q36 and Q37), 12 from the coastal region (9 from Tartus and 3 from Latakia), and Eight dioecious trees of *M. alba* from Tartus (Figure 1, Table 1). All studied trees were morphologically characterized in a previous work (Baroudi, 2017; Baroudi et al., 2023).

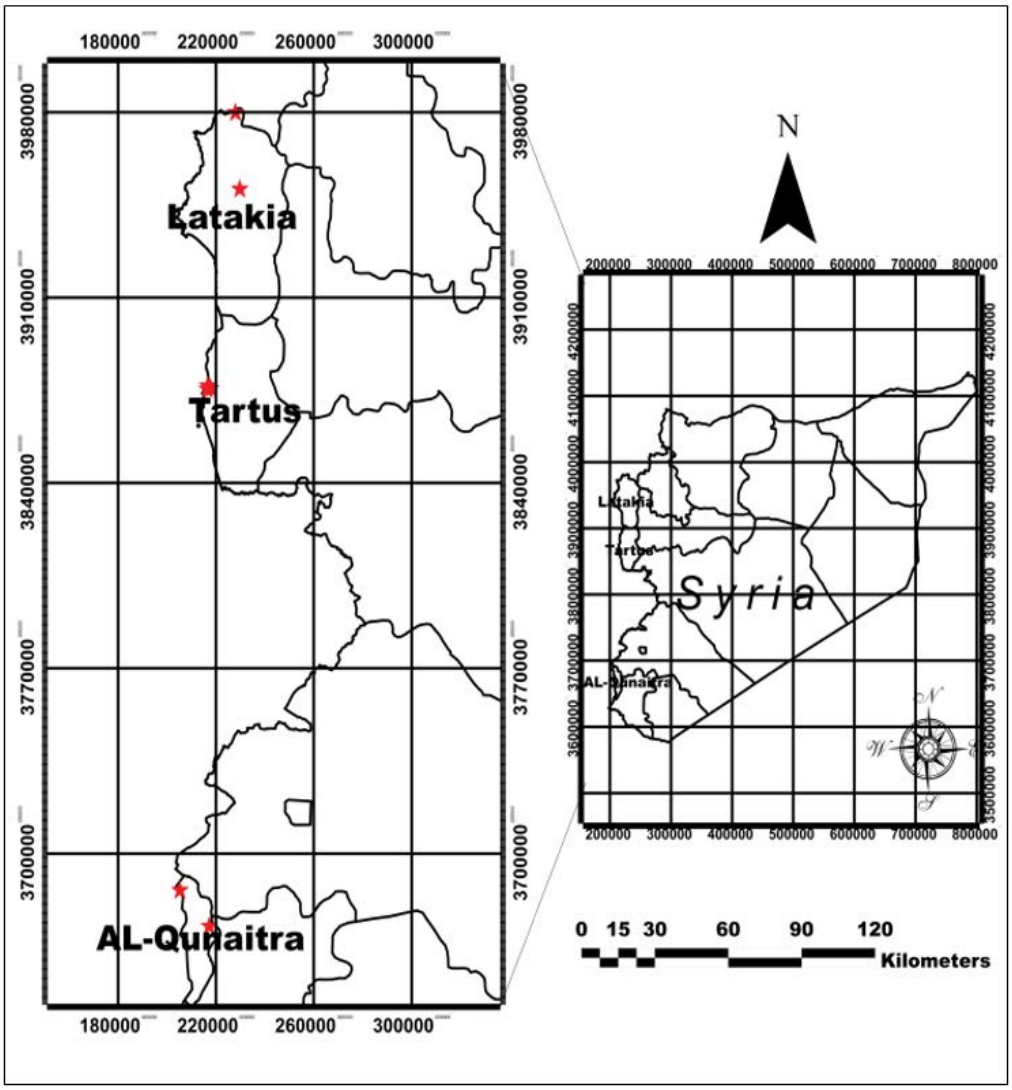

Figure 1. Distribution of studied mulberry genotypes sites in Syria

Table 1. Sample sizes and locations of mulberry genotypes collected in different regions in Syria

NO. Genotypes	Species	Place of collection	Longitude	Latitude
37 (Q1- Q35)	*M. nigra*	Al-Qunaitra (Field)	33◦09′26" N	35◦58′06" E
2 (Q36- Q37)	*M. nigra*	Al-Qunaitra (within another field)	33◦12′36" N	35◦57′24" E
9 (T1- T9)	*M. nigra*	Tartus	34◦59′11" N	35◦54′34" E
8 (M1- M8)	*M. alba*	Tartus	33◦58′58" N	35◦52′21" E
3 (L1- L3)	*M. nigra*	Latakia	35◦40′06" N	36◦04′53" E

DNA extraction

Genomic DNA was extracted from mulberry young fresh leaves using the Cetyl trimethyl ammonium bromide (CTAB) procedure (Doyle and Doyle, 1990). The DNA quantity was visually quantified using the agarose gel electrophoresis. The concentration of DNA was adjusted in all samples and stored at -20 °C until use.

DNA analysis

DNA samples were subjected to Polymerase chain reaction (PCR) amplification with Inter Simple Sequence Repeat (ISSR) primers and Simple sequence repeats (SSR) primer pairs. Amplifications for tested primers and DNA samples were repeated independently three times with the same conditions to verify the reproducibility and consistency of the ISSR and SSR markers.

ISSR analysis

Fifteen ISSR primers were used in this study for the analysis of DNA samples. They were obtained from Metabion International (Germany). The amplification was carried out in 20 µL of reaction mixture containing 1X PCR buffer (10X = 750 mM Tris-HCl. pH 8.8; 0.1% Tween20), 0.2 mM of each dNTP, 2 mM $MgCl_2$, 0.2 µM Primer, 50 ng of genomic DNA and 1 U of DNA *Taq* polymerase (from GeneDireX®). Amplification of DNA was performed in the Eppendorf Master Cycler Gradient System (Eppendorf, Flexlid) following the profile: an initial cycle at 94 °C for 4 min followed by 35 cycles of 94 °C for 30 sec, Ta °C (Table 2) for 30 sec, 72 °C for 2 min and a final extension for 10 min at 72 °C. The PCR products were resolved by electrophoresis on a 1.5% agarose gel in 1 X TBE buffer (89 mM Tris, 89 mM Boric Acid, and 2 mM EDTA), stained by Ethidium bromide (0.5 µg /ml) and photographed with a gel documentation system under UV light.

SSR analysis

Six nuclear SSR primer pairs flanking SSR sequences previously characterized and developed for *Morus* (Aggarwal et al., 2004; Tikader et al., 2009) (Table 2) were used to analyze the DNA samples. Amplification reactions were performed in a final volume of 20 µL in the presence of 1X PCR buffer, 2 mM $MgCl_2$, 0.2 mM of each dNTP, 0.1 µM of each primer pair, 1U of DNA *Taq* polymerase, 50 ng of DNA template. The amplification program consisted of an initial denaturation step at 94 °C for 4 min, followed by 37 cycles of 1 min denaturation at 94 °C, 45 sec at Ta °C (Table 3), and 60 sec. extension at 72 °C with a final extension at 72 °C for 10 min using the thermal cycler used for ISSR analyzes. The PCR amplification products were separated on an 8% polyacrylamide gel and visualized through staining by ethidium bromide.

Table 2. Names, sequence composition and annealing temperatures (ta) for ISSR primers used in the analysis of the mulberry DNA

Primer names	sequence (5' → 3')	Ta °C
UBC-807	AGAGAGAGAGAGAGAGT	47
UBC-808	AGAGAGAGAGAGAGAGC	49
UBC814	CTCTCTCTCTCTCTCTA	47
UBC824	TCTCTCTCTCTCTCTCG	48
UBC825	ACACACACACACACACT	47
UBC-826	ACACACACACACACACC	49
UBC-827	ACACACACACACACACG	49
Mor 02	GTCGTCGTCGTCGTC	57
Mor 09	AGAGAGAGAGAGAGAGTC	51
Mor 10	CTCTCTCTCTCTCTCTGC	52
ISSR10	GACAGACAGACAGACA	45
ISSR13	CTCTCTCTCTCTCTCTGC	53
ISSR18	CTCTCTCTCTCTTG	38
ISSR19	AGAGAGAGAGAGTA	35
ISSR20	CTCTCTCTCTCTCC	41

Data analyses

Bands with high reproducibility were selected for ISSR and SSR polymorphism analysis. The presence and absence of bands (ISSRs) and alleles (SSRs) were coded as one and zero, respectively.

Table 3. Names, sequence composition and annealing temperatures of SSR primer pairs used in the analysis of the mulberry DNA

Primer names	sequence (5' → 3')	Ta °C	GenBank Accession no.
MulSTR1	F: GCCGTGTACCAGTGGAGTTTGCA R: TGACCGTTTCTTCCACTTTACCTAATG	63	AY326440
MulSTR2	F: CGTGGGGCTTAGGCTGAGTAGAGG R: CACCACCACTACTTCTCTTCTTCCAG	65	AY326441
MulSTR3	F: GGGTTGGGTAGATGGGCTTATGTTA R: CCCTATTAACTTTTTGGTCACCTCTA	61	AY326442
MulSTR4	F: GGTCAAGCGCTCCAGAGAAAAG R: GGTGCAGAGGATGAAAGATGAGGT	62	AY326443
MulSTR5	F: CCCCCTGCAATGCCCTCTTTC R: TGGGCGAGGCAGGGAAGATTC	61	AY326444
MulSTR6	F: TCCTTAGGTTTTTGGGGTCTGTTTACAT R: CCTCATTCTCCTTTCACTTATTGTTG	61	AY326445

The numerical Taxonomy and Multivariate Analysis System (NTSYS-pc2.2) program was used to perform the statistical analysis (Rohlf, 2008). The SIMGENE method was selected to estimate the genetic distance between the samples, and data derived using the unweighted pair-group method with arithmetic mean UPGMA algorithm was analyzed by SAHN clustering. The average of genetic diversity per locus (Nei's 1973), Shannon's information index, number of alleles and genetic similarity were evaluated using Popgene software version 1.32 (Yeh et al., 2000). The polymorphism information content (PIC) for each ISSR primer, was calculated using a Power marker program and based on the formula of De Riek et al. (2001).

$$PIC_{(Dominant\ marker)} = 2f\,(1-f),$$

where *f* is the frequency of the present band.

The binary data for 57 mulberry genotypes were also subjected to principal component analysis (PCA) (Gower, 1966) and scores for the first and second components were plotted by past 4.09 analysis software.

RESULTS AND DISCUSSION

Polymorphism and genetic distance evaluated in mulberry using ISSR and SSR markers

The selected 15 ISSR primers amplified a total of 86 DNA fragments in the 57 mulberry genotypes collected from different regions of Syria (figure 2, Table 4). The number of amplified bands per primer ranged between 3 (with ISSR20) to 7 (with Mor02 and ISSR19). Among the 86 fragments, 77 bands showed polymorphism between the samples, with a total polymorphic percentage of 90.31%. The percentage of polymorphic fragments revealed by ISSR primers ranged from 50% [UBC807, (AG)8T, UBC827, (AC)8G)] to 100% [(UBC814, UBC824, UBC 825, UBC826, MOR9, Mor10, ISSR10, ISSR13, ISSR18, ISSR20) (CT)8A, (TC)8G, (AC)8T (AC)8C, (AG)8TC, (CT)8GC, (GA)4 + (CA)4, (CT)8GC, (CT)6TG, (CT)6CC)]. Out of 86 different bands, some unique bands were obtained with some primers (UBC808, UBC825, UBC826, UBC827, ISSR18, MOR09), and could be used to discriminate between *M. nigra* cv. El-Shami and *M. alba* genotypes.

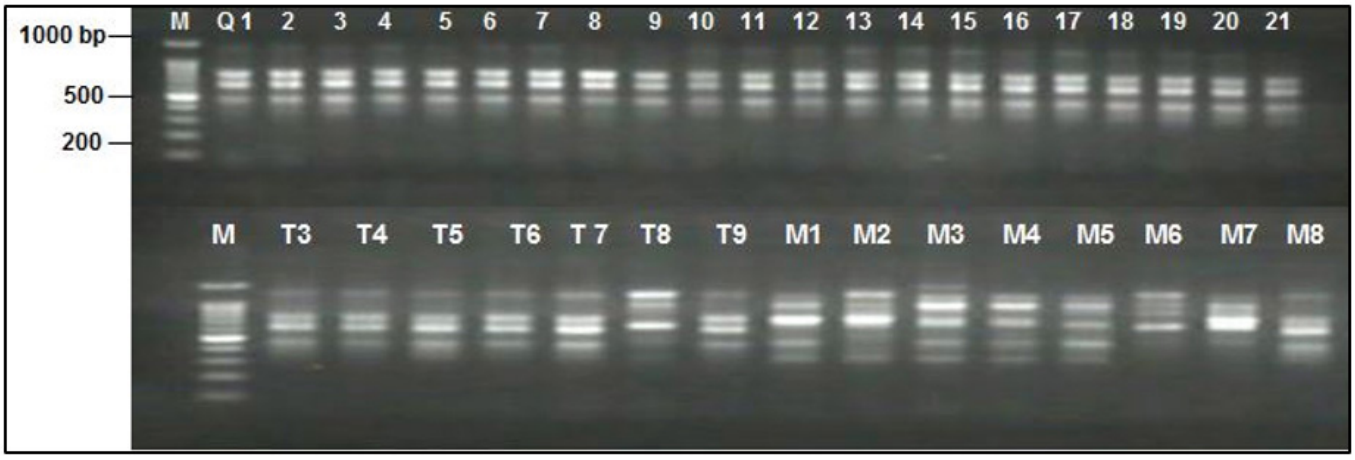

Figure 2. ISSR electrophoretic pattern of DNA amplified by UBC25 (AC)8T Primer (Lane Q1-21: Al-Qunaitra genotypes, lane T3-T9: Tartus genotypes, lane M1-M8: *M. alba* genotypes, M: DNA molecular weight marker in bp)

Table 4. Nucleotide sequences of the 15 ISSR primers, number of amplified and polymorphic bands, % of polymorphic bands and values of genetic diversity

Primer names	sequence (5' → 3')	Number of amplified bands	Number of polymorphic bands	Polymorphic amplified bands (%)	Gene diversity
UBC-807	(AG)8T	6	3	50.00	0.160
UBC-808	(AG)8C	6	5	83.33	0.241
UBC814	(CT)8A	6	6	100.00	0.282
UBC824	(TC)8G	6	6	100.00	0.312
UBC825	(AC)8T	6	6	100.00	0.145
UBC-826	(AC)8C	6	6	100.00	0.216
UBC-827	(AC)8G	6	3	50.00	0.157
Mor 02	(GTC)5	7	6	85.71	0.173
Mor 09	(AG)8TC	6	6	100.00	0.369
Mor 10	(CT)8GC	5	5	100.00	0.143
ISSR10	(GA)4 + (CA)4	4	4	100.00	0.286
ISSR13	(CT)8GC	6	6	100.00	0.369
ISSR18	(CT)6TG	6	6	100.00	0.320
ISSR19	(AG)6TA	7	6	85.71	0.163
ISSR20	(CT)6CC	3	3	100.00	0.471
Mean and total		T = 86	T = 77	M = 90.31	M = 0.254

Six SSR markers (MulSTR1, MulSTR2, MulSTR4, MulSTR5 and MulSTR6) were used to detect variations between the analyzed genotypes (Table 4; Figure 3). The number of alleles observed at a locus ranged from 2 (MulSTR3) to 6 (MulSTR1) with a total of 25 alleles for the six loci and an average of 4.16 alleles/locus.

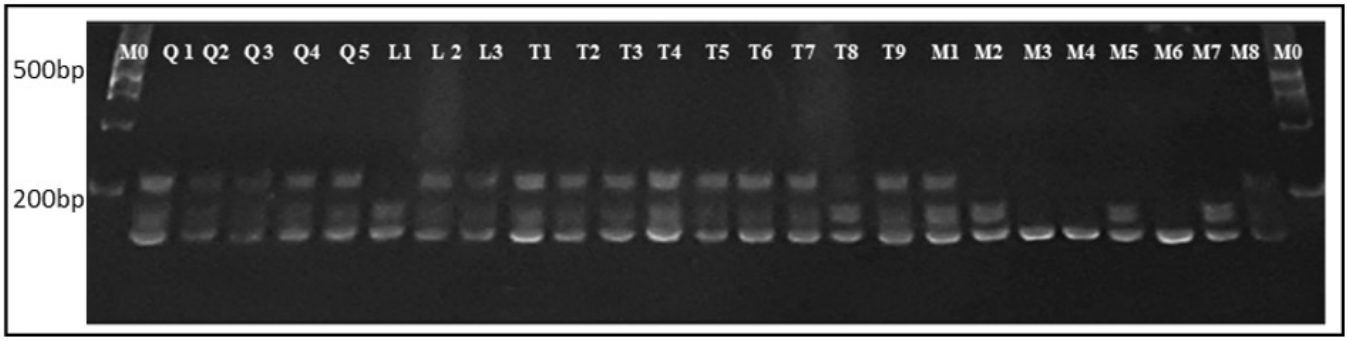

Figure 3. SSR pattern of PCR products amplified using mulstr5 in mulberry genotypes (Lane q1- q5 represent Al-Qunaitra genotypes, lane l1- l3 Latakia genotypes, lane t1-t9 tartus genotypes, lane m1-8: *M. alba* genotypes, lane m0 is DNA molecular weight marker in bp)

The number of polymorphic alleles varied from 2 (MulSTR1, MulSTR2, MulSTR3, MulSTR6) to 5 (MulSTR4) with a total of 16 alleles, and a mean of 2.66 polymorphic alleles/ locus.

In order to evaluate the supportability of markers and to distinguish between the different genotypes, Principal component analysis (PCA) was calculated and the results obtained are represented in Figure 4. The first and the second principal component comprised 35.52% and 13.12% of the total variations, respectively. Variability within the highest Eigen vectors in PC1 and PC2 were as follows: PC1: was strongly associated with (ISSR13, ISSR19, UBC827, MOR09, UBC807), (MulSTR1, MulSTR2, MulSTR4, MulSTR5, MulSTR6). PC2: was strongly associated with (ISSR10, ISSR18, MOR10, UBC808).

The bi-plot axes (Figure 4), generated based on principal components PC1 and PC2 were able to explain more than 48 % of the total variance. PCA analysis for ISSR and SSR data supports their UPGMA clustering (Figure 5).

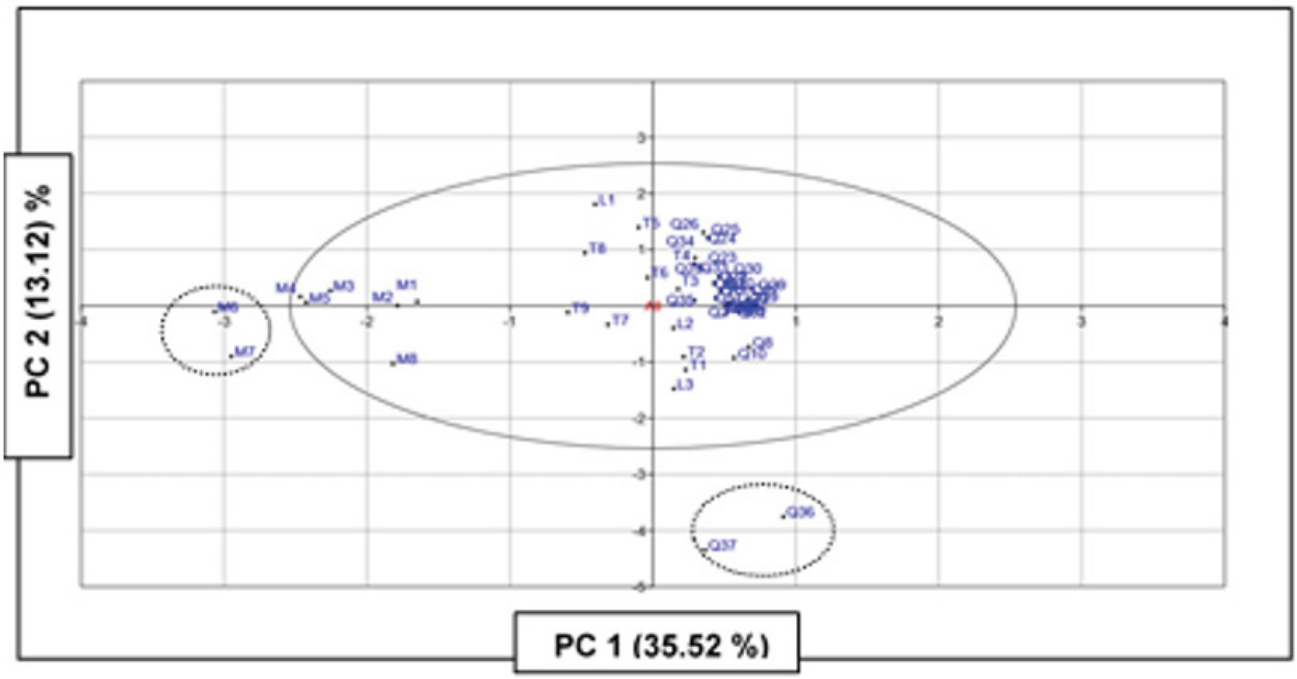

Figure 4. Scatter plot of 57 mulberry genotypes based on first and second components of principal component analysis using ISSR and SSR data (q1-q35 (q36, 37) Al-Qunaitra genotypes, t1-t9: Tartus genotypes, m1- m8: *M. alba* genotypes)

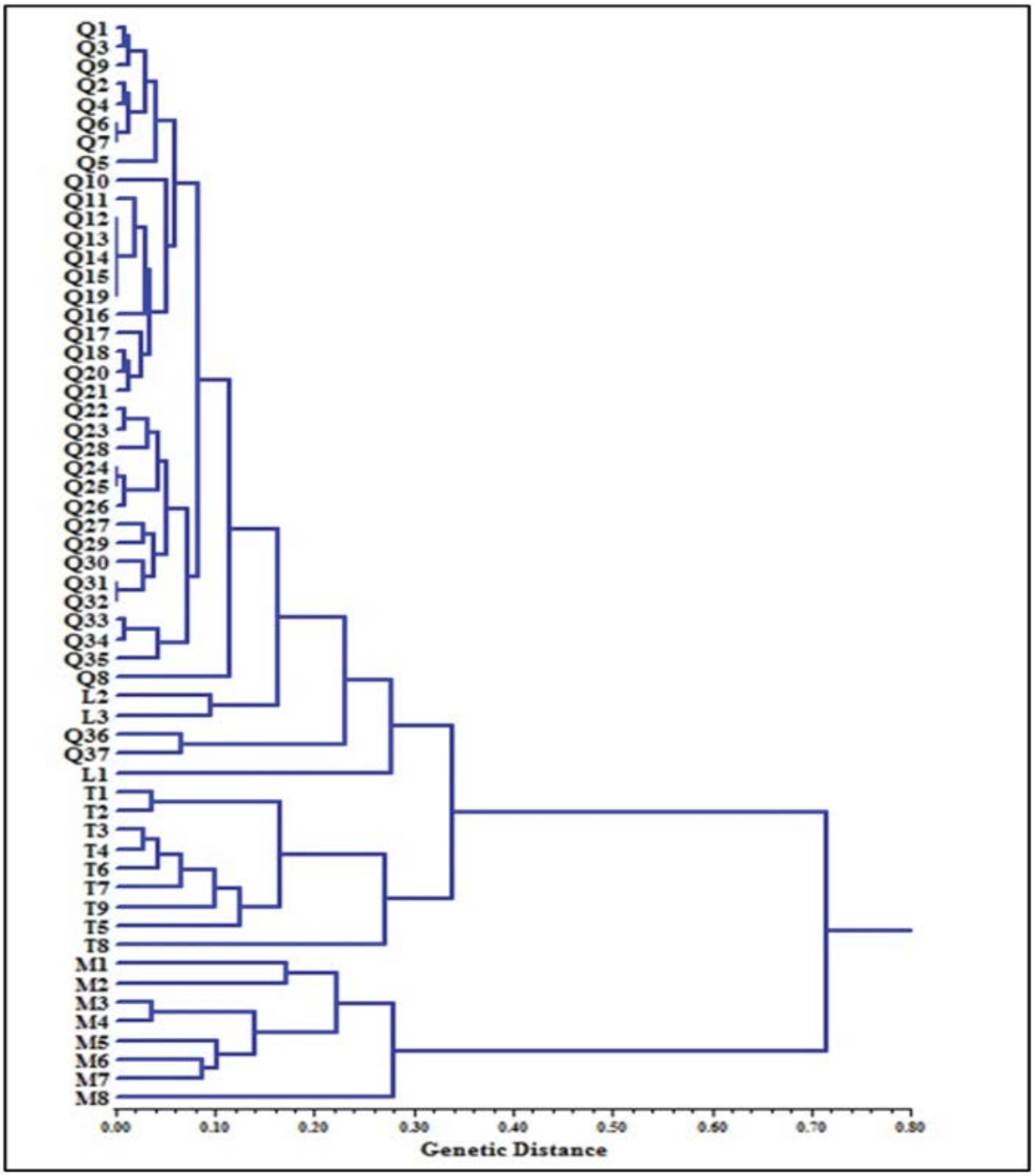

Figure 5. A dendrogram representing the coefficient of genetic distance between 57 mulberry genotypes, based on ISSR and SSR marker results

Genetic distance and genetic diversity between and within Morus species

The presence and absence of bands (ISSR) and alleles (SSR) were used to establish the matrix used to estimate the genetic distance between the different samples and to create a dendrogram showing the relationship between them (Figure 5).

In the dendrogram, samples were clustered in two distinct branches with a 0.70 value of genetic distance coefficient. The first branch includes all mulberry samples belonging to *M. alba* (M1 to M8) and the second one contained those from *M. nigra*. The highest value of genetic distance coefficient (0.28) in the first branch existed between the sample M8 and the samples M1

and M2, while this value was 0.03 between the closest samples (M3 and M4). All genotypes from *M. alba* showed variations in molecular patterns, no identical genotypes were detected, but the genetic distance values between them were low, reflecting the high level of genetic similarity within the species.

The second branch comprising *M. nigra* samples was also divided in two distinct groups with a 0.33 genetic distance coefficient. The first group contained samples collected from Tartus (T1 to T9), they were all different but with low genetic distance values. The second group contained samples collected from Latakia (L1, L2 and L3) and Al-Qunaitra (Q1 to Q37). Many samples from Al-Qunaitra were identical but dispersed in different clusters [(Q12, 13, 14, 15, and 19) (Q24 and 25) and (Q31 and 32)].

In general, the samples from Al-Qunaitra showed a high level of similarity where the genetic distance coefficient between all samples (Except Q36 and Q37) was less than 0.1, this could be explained by the fact that all these samples were collected from one field and could have the same origin, while the distinct samples (Q36 and Q37) were collected from a distant mulberry field and may have different resource.

Only samples from Latakia showed higher diversity and were dispersed along the dendrogram. This difference could be explained by the fact that the samples were collected from individual trees dispersed in distant areas from Latakia. The high level of genetic similarity between *M. nigra* genotypes was also observed in the study of Wang et al. (2017), where low diversity among 42 mulberry genotypes was revealed using 17 ISSR primers (average of Nei's and Shannon's information index 0.116 and 0.174, respectively).

The genetic relationships represented by the dendrogram (Figure 5) showed a high correlation between the samples and their collection areas (except for the two samples Q36 and Q37), where samples from each region were clustered together in a separate cluster. Similar results were obtained in the study of Zhuowei et al (2001) when genotypes were regrouped according to the collection areas using the AFLP marker. In the contrary, Sharma et al. (2000) using AFLP, and Vijayan et al. (2004) using ISSR on mulberry genotypes, from different species and geographical locations, reported a lack of clear relationships between geographical origin and genetic similarity.

According to their results, this lack is due to the introduction followed by the naturalization of mulberry genotypes in areas away from their initial origin. Similar results were found by Eun-Ju et al. (2020), they used ISSR markers and found high level of genetic similarity (coefficient ranged between 0.67 and 0.99) between berry genotypes and no correlation between molecular data and collection regions.

The dendrogram of this study showed that the coefficient of genetic distance between species *M. alba* and *M. nigra* was high (0.72), whereas within the species it was around 0.3. This information was in agreement with another study applied on *M. nigra* and *M. alba* species in the northwestern region of Syria using AFLP marker and suggested higher interspecific genetic distance than intraspecific distance (Abed-Elkader, 2014), and the same results were also proved by Un-Hyang et al. (2021).

The analysis of genetic diversity in the *Morus* species used in this study showed low levels of variations within and between species. The value of genetic diversity detected in *Morus* varied depending on the molecular marker used in its estimation. These values ranged from 0.14 (UBC825, MOR10) to 0.47 (ISSR20) with an average of 0.25, when ISSR primers were utilized (Table 4), while they ranged between 0.07 (MulSTR1) and 0.26 (MulSTR3) and with an average of 0.14 where SSRs marker were used (Table 5).

In order to understand the distribution of genetic diversity in mulberry samples and to know whether the geographic regions affected its level, the samples were regrouped in three ways and analyzed with the Popgene program. The first analysis (G1) included all genotypes analyzed (*M. nigra* and *M. alba*) from the different regions, and the second way (G2) in relation to the collection site and species, the samples were divided into 4 groups, 3

Table 5. Names of SSR loci, number of total alleles, number of polymorphic alleles, gene diversity, and polymorphic information content (pic) values observed in mulberry genotypes

Primer names	Repeat moti	Number of alleles	Number of polymorphic alleles	Polymorphic alleles %	Gene Diversity	PIC
MulSTR1	(GTT)6 + (GTT)4	6	2	33.33	0.071	0.06
MulSTR2	(GTT)11	4	2	50	0.08	0.07
MulSTR3	(GA)33	2	2	100	0.26	0.23
MulSTR4	(GAA)6	5	5	100	0.24	0.24
MulSTR5	(CCA)8	4	3	75	0.11	0.10
MulSTR6	(GT)15	4	2	50	0.09	0.08
Mean and total		T = 25	M = 2.66	M = 68.055	M = 0.14	M = 0.13

groups of *M. nigra* (samples from Al-Qunaitra, Tartus and Latakia), and one of *M. alba* from Tartus. In the third way (G3), samples were distributed in two groups according to the species, one included all samples belonging to *M. nigra* and the other contained samples of *M. alba*. Genetic diversity among and between samples included the analyses of Nei's gene diversity (h), and Shannon's information index (I) (observed and effective number of alleles (Table 6).

The value of genetic diversity of overall samples in G1 was 0.2202, this value varied when the genetic diversity was estimated for samples from each region (in G2). The highest values (0.1481 and 0.1197) were detected in Tartus samples (in *M. alba* and *M. nigra*, respectively), and the smallest value (0.0757) was revealed in Al-Qunaitra samples.

Similar results were obtained when Shannon's Information index was calculated, it was 0.35 for G1 samples, while within G2 Tartus genotypes (*M. alba*) had the highest value 0.22 followed also by *M. nigra* in Tartus (0.1828). When the genetic diversity calculated at the species level (In G3), the value was 1.7072 in *M. nigra* and 1.4144 in *M. alba*.

The percentage of polymorphism detected in the mulberry genotypes in G1 was 83.78%, but this value was smaller when it was calculated for each group in G2, where it was 36.94% in Al-Qunaitra, 36.04% in Tartus, 24.32% in Latakia, 41.44% in *M. alba* in Tartus. Different polymorphism values were found when the evaluation was based on species level in G3, where these values were 70.27% in *M. nigra* and 40.41% in *M. alba* (Table 6).

An effective number of alleles for overall populations was 1.33, considering that Tartus genotypes (*M. alba*) had the largest number of effective alleles 1.24. Through the previous analyses, it was clear that *M. nigra* is molecularly well distinct from (*M. alba*) as the genotypes were clustered in two distinct clusters in Figure 4. A similar result was obtained by Kafkas et al. (2008) when they were able to discriminate molecularly between *M. nigra*, *M. rubra* from *M. alba* and in Un-Hyang et al. (2021) and in Zhao et al. (2007) where their study on cultivated and wild mulberry genotypes using ISSR and SSR markers revealed a clear distinction between wild and domesticated species.

The analysis of genetic diversity showed a low level of genetic diversity in the whole collection but this level was slightly higher in *Morus alba* (0.148) compared to *Morus nigra* (0.145), despite the difference in the number of samples between the two species (49 in *M. nigra* and 8 in *M. alba*). The low genetic diversity in *M. nigra* was also revealed by Wang et al. (2016) using 42 mulberry genotypes where the values of Nei's and Shannon's information index were 0.116 and 0.174, respectively.

Our results agreed with the results of Abed-Elkader (2014) who observed a higher level of genetic diversity in *Morus alba* compared to *Morus nigra*, and with Orhan et al., (2020) where they suggested that white mulberry

Table 6. Values of genetic diversity for 57 mulberry genotypes, overall (group 1), with respect to the location and species (group 2) and among both species (group 3)

The Groups		Genotypes	The observed number of alleles	The effective number of alleles	Nei's (1973) gene diversity	Shannon's Information Index (I)	The number of polymorphic loci	The percentage of polymorphic loci %
G1	Overall	57	1.8378	1.3384	0.2202	0.3500	93	83.78
G2	Al-Qunaitra	37	1.3694	1.1166	0.0757	0.1246	41	36.94
	Tartus	9	1.3604	1.1952	0.1197	0.1828	40	36.04
	Latakia	3	1.2432	1.194	0.1081	0.1548	27	24.32
	(Tartus) *M. alba*	8	1.4144	1.246	0.1481	0.2227	46	41.44
G3	Al-Qunaitra, Tartus, Latakia	49	1.7027	1.2189	0.145	0.2387	78	70.27
	(Tartus) *M. alba*	8	1.4144	1.246	0.1481	0.2227	46	41.44

genotypes had the highest genetic diversity comparing to the red and black mulberry genotypes collected from different regions in Turkey. The results of Srivastava et al. (2004) showed that although *M. alba* is more diverse than *M. nigra* but still has a high level of genetic similarity (range of 0.738 to 0.909 with an average of 0.834).

On the contrary, Kadri et al. (2021) found a high level of genetic diversity within 70 genotypes of different mulberry species from different geographical regions in Lebanon using the combination of morphological and molecular markers (SSR and ISSR).

It is important to know that El-Shami mulberry genotypes (*M. nigra*) have become cultivated and propagated mainly by clonal growth in Syria, and these local genotypes were domesticated by long-term cultivation. These characteristics may be responsible for the low diversity between their genotypes, that is agree with Zhao et al. (2007).

CONCLUSION

The genetic diversity detected in *M. nigra* cv. El-Shami genotypes analyzed in this study showed a high level of genetic similarity within the species especially between genotypes collected from Al-Qunaitra, and it was lower than between the samples of *M. alba* genotypes which showed variations in molecular patterns. That could result of the agronomical habits used in that area. Therefore an additional study including different regions of Syria will be needed to estimate the real genetic diversity in *Morus* and to be able to draw a strategy to increase and conserve the genetic diversity of the species.

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