

Molecular characterization and relationships among some fig (*Ficus carica* L.) genotypes from the collection of the Faculty of Horticulture, Bucharest, using simple sequence repeat (SSR) markers

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ABSTRACT

The development of molecular marker technology in the late 20th century has revolutionized plant breeding, particularly for minor species that previously lacked sufficient data. Accurate information on structure and genetic diversity is crucial to improve this activity. This study was conducted on fig accessions from various regions of Romania. The accessions are part of the fig germplasm collection at the Faculty of Horticulture, Bucharest. The genetic variability among 31 fig (*Ficus carica* L.) accessions was examined using 17 simple sequence repeat (SSR) markers previously developed for this crop. The primer pairs revealed a total of 54 alleles. The number of alleles per locus ranged from two to six, with an average of 3.60 alleles per locus. The mean expected (H_e) was 0.55, and the mean polymorphic information content (PIC) was 0.61. The genetic relationships are illustrated by hierarchical clustering. UPGMA cluster analysis grouped the fig accessions analyzed into two principal clusters. All of these suggest a molecular diversity among the studied fig accessions.

Keywords: Accessions, alleles, cluster, germplasm, microsatellites.

INTRODUCTION

Figs, the fruits of *Ficus carica* L. ($2n=2x=26$) from the *Moraceae* family, are among the oldest domesticated fruit species, before millet and wheat (Kislev *et al.*, 2006). This may be due to the easy propagation of the fig tree, which is generally done through cutting (Zohary and Hopf, 2000). It is believed that fig originated from the Middle Asia and the Mediterranean Basin (Condit, 1955). However, it is now also cultivated in other temperate countries (Sadder and Ateyyeh, 2006). The fig tree belongs to the genus *Ficus*, a large group that includes 600 to 1,900 species (Flaishman *et al.*, 2008).

The fig tree grows easily in warm and temperate climates, being a xerophilous plant (Minone *et al.*, 2011). It is tolerant to drought (Ammar *et al.*, 2022), does not require rich soils, and is resistant to pests (Jullien and Jullien, 2011).

Figs are consumed both as fresh and especially dried fruits (Duenas *et al.*, 2008), being very perishable fruits due to fungal attack after harvest (Dogan *et al.*, 2018). Fig trees are cultivated on 290,000 ha worldwide (FAOSTAT, 2019). Turkey is the world's main producer of figs (Veberic and Mikulic-Petkovsek, 2016).

The fruits of *Ficus* species are reported to represent an important source of essential medicinal and dietary nutrients, among which figs, the fruits of *Ficus carica*, are the most used (Shi et al., 2018). Consumption of figs has positive effects on human health (Khatib and Vaya, 2010). This is based on their content in amino acids (Saki et al., 2019), vitamins, fibers, mineral, carbohydrates (Hssaini et al., 2019 and Hssaini et al., 2020a), their antioxidant capacity (Arvaniti et al., 2019), their polyphenolic compounds such as flavones, quercetin, and rutin (Pourghayoumi et al., 2017), and other nutraceutical qualities (dos Anjos Cruz et al., 2022).

The first characterizations of this species relied on agronomic and morphological traits, following the descriptors developed by IPGRI and CIHEAM (2003).

Although numerous descriptors exist, a standardized method for fig characterization is still lacking. Generally, figs are characterized by region, and environmental conditions strongly influence the morphological and biometric traits, which makes it difficult to compare genotypes, especially if they are very similar. Morphological characterizations of some fig genotypes were made in Slovenia (Podgornik et al., 2010), Tunisia (Gaaliche et al., 2012), Iran (Khadivi et al., 2018), Algeria (Mahmoudi et al., 2018), Morocco (Hssaini et al., 2020b), and for male fig (caprifig) in Iran (Khadivi-Khub and Anjam, 2014; Mirheidari et al., 2020), and Tunisia (Essid et al., 2017).

The development of molecular marker technology in recent years has significantly improved precision in plant breeding (Nadeem et al., 2018). Examples of molecular markers include microsatellite or Simple Sequence Repeat (SSR) data, Restriction Fragment Length Polymorphism (RFLP) data, and Single Nucleotide Polymorphism (SNP) data. Due to their high degree of polymorphism, reproducibility, and codominance, SSR (microsatellite markers) are most commonly used to create a genetic fingerprint for most plant species (Gupta and Varshney, 2000). A good genetic characterization depends on the high quality of the extracted DNA (Armiteye, 2021). For fig, among the first research where microsatellite markers (SSR) were used, we find Khadari et al., 2001; Giraldo et al., 2005; Giraldo et al., 2008; Bandelj et al., 2007, but also more recent studies, Costa et al., 2017; Ferarra et al., 2017; Marcotuli et al., 2019; Ergul et al., 2021; Essid et al., 2021.

The main objective of this study was to characterize and assess the genetic diversity of fig genotypes from the collection of the Faculty of Horticulture, in Bucharest, using SSR markers to support the development of conservation strategies for the endangered genetic resources of this species.

MATERIALS AND METHODS

Plant material

This study was conducted on 31 fig accessions with diverse phenotypic traits from various Romanian regions (Table 1). All accessions are part of the fig germplasm collection of the Faculty of Horticulture, Bucharest. The plant material was propagated through hardwood cuttings.

Table 1. List of genotypes of collection and their origin area

Genotype	Location	Genotype	Location	Genotype	Location	Genotype	Location
4 i	Bucharest	241 cv	Lunca	106 m	Popești-Leordeni	184 cv	Viisoara
10 i	Mangalia	4 m	Bucharest	125 m	Moara Domneasă	185 cv	Tulcea
16 ia	Bucharest	4 ma	Bucharest	19 L	Bucharest	187 cv	Mangalia
100 i	Bucharest	4 mb	Bucharest	28 L	Brazi	189 cv	Tulcea
103 i	Săvârșin	11 m	Giurgiu	50 L	Săvârșin	266 cv	Constanța
109 i	Svinița	19 m	Bucharest	183 L	Alba-Iulia	267 cv	Craiova
161 i	Svinița	21 m	Bucharest	207 L	Croatia	275	Snagov
125 i	Moara Domneasă	63 m	Moara Domneasă	181 cv	Voiteg		

Genomic DNA extraction

Genomic DNA was extracted from 25 mg lyophilized and fine-powdered winter dormant buds following a cytrimethylammonium bromide CTAB-based method (Futterer *et al.*, 1995) with modifications. The concentrations of DNA were determined by using a Quibit fluorometer (Invitrogen, USA) and diluted to 50 ng/μL.

SSR analysis

A total of 17 SSR primer pairs, previously developed by Giraldo *et al.* (2005), were used for SSR analysis in this study (Table 2). Amplification reactions were performed in a final volume of 20 μL containing of 50 ng of template DNA, 1X reaction buffer, 1.0 U Taq DNA polymerase (Thermo Scientific, USA), 0.10 μM M13 sequence-tailed forward primer, 0.20 μM reverse primer, 0.20 μM M13 primer labeled with infrared dye at either 700 nm or 800 nm (Licor, USA) and 0.25 mM dNTPs. PCR reactions were conducted using a Veriti 96-well thermal cycler (Applied Biosystems, USA). PCR conditions were as follows: 2 min at 94°C; 6 cycles of 40s at 94°C, 1 min at 61°C (with the annealing temperature reduced 1°C after every cycle for touchdown PCR protocol), 1 min and 30s at 72°C; 28 cycles of 40s at 94°C, 1 min at 55 °C and 1 min and 30s at 72°C; 7 cycles of 40s at 94°C, 1 min at 54°C and 1 min and 30s at 72°C and final extension step of 10 min at 72°C. The PCR products were separated on 6.0% polyacrylamide gels at 30 W for 2 to 3 h using a Li-COR 4300 DNA analyzer (LI-COR).

Data analysis

A genetic similarity matrix of fig accessions was calculated using a Dice coefficient (Dice, 1945) and the NTSYSpc v2.21 program (Exeter Software, New York, USA), based on the presence (1) and absence (0) of the SSR primers. Genetic distance among accessions was determined using the unweighted pair group method with arithmetic mean (UPGMA). The method of Levene (1949) and the POPGEN32 software v.1.32 (Yeh *et al.*, 1997) were used to calculate expected heterozygosity (He) and observed heterozygosity (Ho). The polymorphism information content (PIC) values for SSR markers were calculated according to the method of Botstein *et al.* (1980). Population structure was analyzed using the Structure v.2.3.4 software, based on a Bayesian clustering model. (Pritchard *et al.*, 2000). The range of possible numbers of clusters (K) varied from 1 to 12, with 5 replications for each K value. Each independent run was carried out with a burn-in period of 100,000 steps followed by 100.000 Monte Carlo Markov Chain (MCMC) replications. Furthermore, the method defined by Evanno *et al.* (2005) was used to detect the most likely number of subpopulations (K).

Table 2. Genetic diversity parameters assessed for 17 SSR loci in 31 fig genotypes

Locus	Na	Ho	He	PIC
LMFC 12	monomorphic	-	-	-
LMFC 13	5	0.29	0.66	0.86
LMFC 14	3	0.35	0.54	0.61
LMFC 15	4	0.77	0.66	0.60
LMFC 17	3	0.68	0.63	0.57
LMFC 19	4	0.90	0.74	0.64
LMFC 21	3	0.34	0.40	0.54
LMFC 22	4	0.38	0.54	0.58
LMFC 25	3	0.48	0.37	0.55
LMFC 26	4	0.48	0.39	0.59
LMFC 27	2	0.37	0.50	0.57
LMFC 28	monomorphic	-	-	-
LMFC 30	6	0.83	0.79	0.75
LMFC 32	5	0.38	0.46	0.66
LMFC 35	3	0.74	0.55	0.66
LMFC 37	2	0.41	0.37	0.56
LMFC 40	3	0.74	0.70	0.54
Mean	3.60	0.54	0.55	0.61

Na – number of different alleles; Ho – observed heterozygosity; He – expected heterozygosity; PIC – polymorphism information content

RESULTS AND DISCUSSIONS

A set of 17 SSR markers was used to assess genetic distance among the 31 fig accessions. It was found that 15 SSRs were polymorphic; meanwhile, 2 of them were monomorphic (LMFC 12 and LMFC 28). LMFC 12 was also found monomorphic by Teoman *et al.* (2017), but polymorphic by Costa *et al.* (2017) with 6 alleles, Giraldo *et al.* (2005) with 3 alleles. LMFC marker was found to be polymorphic by Giraldo *et al.* (2005) with 4 alleles, Costa *et al.* (2017) with 6 alleles, Teoman *et al.* (2017) with 5 alleles, and Essid *et al.* (2021) with 5 alleles. 15 SSR markers amplified 54 polymorphic SSR alleles, and the number of polymorphic alleles per SSR marker ranged from 2 (LMFC-27 and LMFC-37) to 6 (LMFC-30), with an average of 3.60 (Table 2). A higher number of alleles for LMFC 30 was also reported by Ergul *et al.* (2021) with 9 alleles and Essid *et al.* (2021) with 6 alleles. The observed (H_o) values for each SSR marker ranged from 0.29 (LMFC-13) to 0.90 (LMFC-19) with an average of 0.54, while the expected (H_e) heterozygosity varied from 0.37 (LMFC-25 and LMFC-37) to 0.79 (LMFC-30) with an average of 0.55 (Table 2). Comparing both H_o and H_e values of the primer pairs, a heterozygote excess ($H_o > H_e$) was observed for LMFC-15, LMFC-17, LMFC-19, LMFC-25, LMFC-26, LMFC-30, LMFC-35, and LMFC-40, whereas a heterozygote deficiency ($H_o < H_e$) was observed for the remaining SSR markers.

The polymorphism information content (PIC) value ranged from 0.54 (LMFC-21 and LMFC-40) to 0.86 (LMFC-13), with a mean of 0.61. These results indicated that all loci were highly informative ($PIC > 0.50$), and the most powerful locus for discriminating fig genotypes was LMFC-13. $PIC = 0.86$ for LMFC-13 was higher than $PIC = 0.42$ found by Costa *et al.* (2017), but lower than $PIC = 0.90$ found by Teoman *et al.* (2017).

The number of gene pools indicated by the STRUCTURE analysis for these 31 fig genotypes was estimated to be $K = 2$ (Figure 1), with the highest ΔK value of 6.36. As shown in Figure 2, gene pool 1 contained 17 genotypes collected from the Southwestern part of the country (Svinița, Săvârșin) - 5, Bucharest - 4, Constanța - 3, Teleorman - 1, Alba - 1, Prahova - 1, Snagov - 1, Croatia - 1, while gene pool 2 consisted of 14 genotypes collected from Bucharest - 10, Tulcea - 3, Craiova - 1. It can be observed that the genotypes from the Bucharest area were present in both gene pools, although about 71% of genotypes in gene pool 2 originate from Bucharest. Even the genotypes from the Dobrogea area are found in both gene pools, but they are well separated. The genotypes from the southeastern part of Dobrogea (Constanța) are all in gene pool 1, while the genotypes from the northern part of Dobrogea (Tulcea) are in gene pool 2. Similarly, it was observed that 47 caprificus genotypes were placed in two gene pools based on STRUCTURE analysis (Teoman *et al.* 2017).

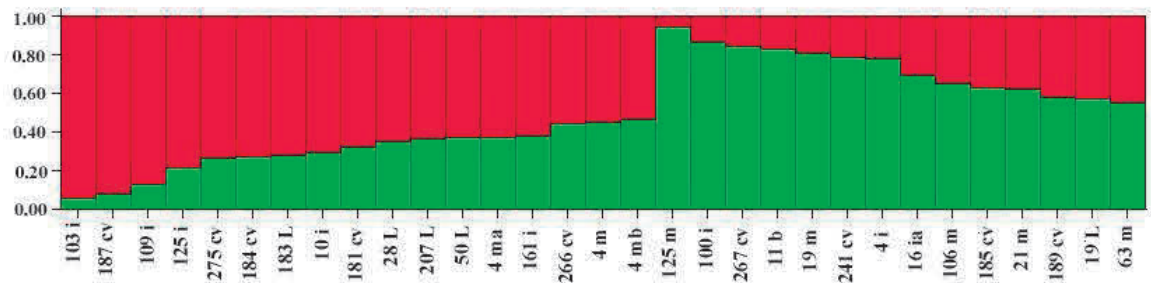


Figure 1. The STRUCTURE bar graph based on 15 SSR markers. Different colours represent different gene pools

According to the UPGMA dendrogram, the 31 fig genotypes were mainly divided into two distinct clusters (A and B), which correspond to the two gene pools identified by STRUCTURE analysis for $K = 2$ (Figure 2). Each cluster was further separated into two

subgroups: Cluster A into Group I and Group II, and Cluster B into Group III and Group IV. Genetic similarity among the fig genotypes ranged from 0.34 to 0.87, indicating high genetic variation in this study. The highest similarity was observed between the genotypes 185 cv and 187 cv (0.87), while the lowest similarity was recorded between 184 cv and 125 m genotypes (0.34).

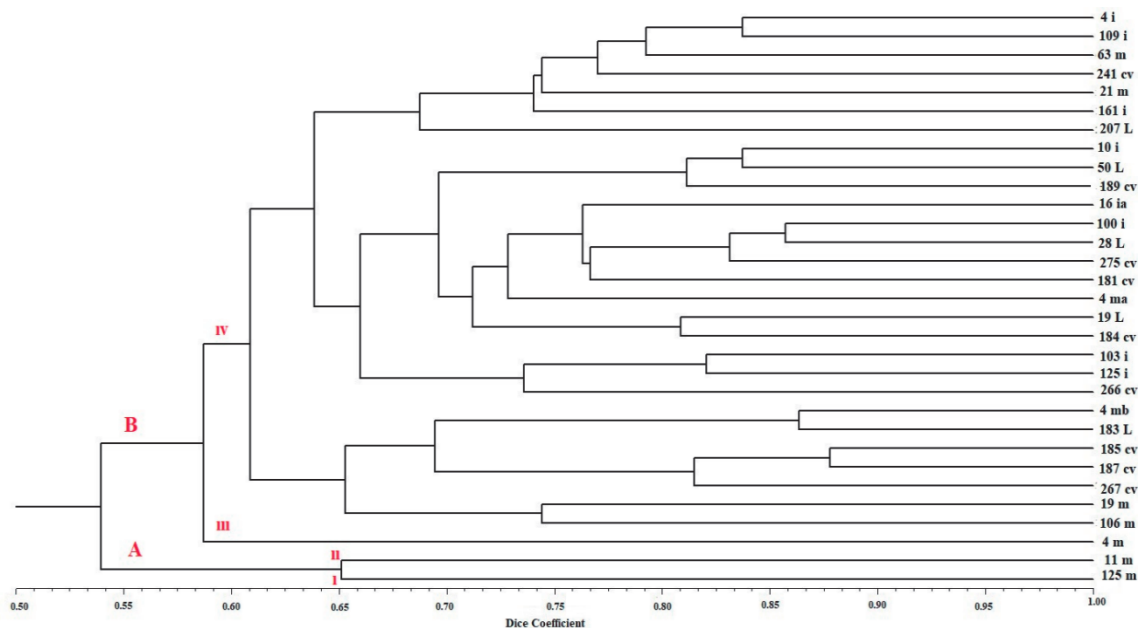


Figure 2. UPGMA dendrogram based on the Dice similarity of 15 SSR markers showing the genetic relationships among 31 fig genotypes.

CONCLUSIONS

The present research demonstrated that all studied fig genotypes from the collection of the Faculty of Horticulture in Bucharest, are genetically distinct. Additionally, there were no homonyms or synonyms among the genotypes in this study, indicating that all genotypes are unique. Genetically related genotypes may share a common genetic origin; however, they are influenced by external factors such as the environment in which they are cultivated. Furthermore, the high genetic variation between the fig genotypes will be valuable for developing national fig breeding programmes. The results of this study also highlight that SSR analysis is an efficient tool to identify fig genotypes.

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