2023 MULTIDISCIPLINARY CONFERENCE ON SUSTAINABLE DEVELOPMENT
Timisoara, 25-26 May

Analysis of Romanian Fig tree cultivars (Ficus Carica L.) using RAPD markers

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Abstract: *In the present study, we investigated the genetic diversity, of 9 local Romanian fig cultivars using RAPD analysis. From the RAPD primers used, six primers generated polymorphic bands ranging between 81.81 % in case of OPG -02 and 100% for the oligonucleotides OPA-05. The polymorphic bands, registered per primer ranged from 6 (primer OPA-10) to 11 (primer OPG-02). Based on the RAPD analysis using these primers, we see that there is a high genetic variability between studied cultivars of Ficus carica, which can be exploited effectively in the improvement programs for this species. An appropriate genetic variability in hybrid populations can be obtained using the cultivars from various clusters and subclusters. The cultivars of Ficus carica tested were characterized by a large variance at the DNA level. Results from the study confirm that RAPD markers can be used in studies concerning genetic variability in Ficus carica.*

• Introduction

In human history the fig tree (*Ficus spp*) is known as one of the oldest fruit trees. It is a member of the Moraceae family . All over the world fig fruits are eaten fresh or dry, having high nutritional value. Figs are also known for their mild laxative activity. The fig tree produces a single crop in the fall, or it may bear fruit twice: in the spring (fig flowers), then in the summer and fall (autumn figs). Its importance worldwide is likely to continue due to its nutritional value and health benefits. Figs are an excellent source of minerals, vitamins and amino acids, contain the highest concentration of polyphenols, and contain more crude fiber than other common fruits and drinks. In general, three genetic markers are used for the characterization and identification of different fruit species. These markers are : morphological or visible markers, biochemical variants or isozymes, and molecular markers. New genetic markers based on DNA polymorphisms have been developed for genetic diversity analysis and cultivar identification between and within the fruit species . The assessment of genetic relatedness and diversity has been investigated using RFLP, AFLP, SSR, ISSR, and RAPD methods. Compared with other molecular techniques, RAPD is a simple, fast, efficient, and inexpensive method. Additionally, it is not necessary to know the sequences in markers and it can produce abundant polymorphic fragments. In the last decade, polymerase chain reaction-based RAPD technology has become one of the most commonly used molecular techniques for DNA marker development. Unlike conventional PCR analysis, RAPD does not require specific knowledge of the DNA sequence of the target organism: depending on where it is complementary to the primer sequence, the same 10-mer primer will or will not amplify a stretch of DNA. If the primers anneal too far or if 3' ends of the primers do not face each other, many fragments will be generated. Therefore, RAPD has become a powerful and accurate tool for analyzing genetic relatedness and diversity in figs. Plant characterization using molecular markers is an ideal method for improving and conserving plant genetic resources. This work aimed to analyze the genetic diversity of fig from western Romania using (RAPD) markers.

• Material and method

Sample collection-Leave samples of Ficus carica were collected from the western part of Romania (Svinita, Timisoara and Sebis).

Extraction of DNA-Genomic DNA was extracted from 50 mg of fresh young leaves using a DNA easy Plant Mini Kit with some modifications. 9 RAPD primers from (Operon Technologies, Alemada, USA) were used for PCR amplification.

RAPD - PCR reaction

DNA amplification was performed using a Corbett thermal cycler. The following PCR program was used, which includes an initial DNA denaturation step at 94°C for 5 minutes, followed by 30 cycles of 92°C for 30 seconds,40 °C for 30 seconds and 72 °C for 90 seconds. Includes a final extension of 5 minutes at 72°C

Agarose gel Electrophorosis: The RAPD products obtained were separated by electrophoresis at 3 V cm-1 in gels of agarose 2%, which were run with the 1xTAE buffer. The gel was colored with ethidium bromide, after which the DNA bands were visualized with a UV transilluminator using a photo image system. A PCR marker was used as a molecular weight standard (1000-50bp) which was also run on each gel.

Data Analysis:

The images of the gel were analyzed using Photo capt Mw software. After the analysis performed with different primers, only clear bands (1) were taken into account, and those with very low resolution were noted as absent (0). The clear bands were entered into a binary matrix. Some parameters were calculated to evaluate the interpopulation variability of the studied genotypes,

-The polymorphism total (PIC) :

$$PIC = 1 - \sum_{i=1}^{n} P_{ij}^2 - \sum_{i=1}^{n-1} \sum_{j=i+1}^{n} 2P_i^2 P_j^2$$

Pi- allele' frequency; Pij- frequency allele i for locus j; Pj- allele' frequency;
The n - total number of loci.

-The index of discrimination (Pi).

$$PI = \sum PIC$$

The Jaccard coefficient was used to establish the genetic similarity between the studied genotypes.

JC= a/(a+b+c), where a, b, c represent the commons and uncommonness of those genotypes [21].

The dendrogram was constructed on the basis of the matrix of genetic similarity using the cluster media method.

• Results and discussions

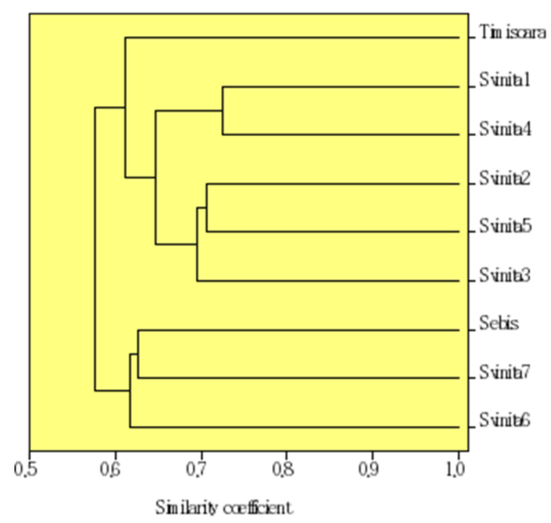
To analyze the genetic polymorphism of fig cultivars, nine RAPD primers were surveyed. Following the RAPD analysis of the 9 varieties of Ficus carica, a large number of distinct fragments were produced for each primer. A total of 51 detectable bands were generated by the 6 primers, 45 (88.23%) of these were polymorphic, with an average of 8.5 amplicons/primer. Primer OPG-02 produced the most fragments (11 amplicons), while primer OPA-10 recorded the lowest number (6 amplicons).

From the data presented it can be noted the presence of the genetic diversity among the analyzed cultivars. Our study showed a high polymorphism of 91.91% (RAPD marker) among the cultivars examined. Total polymorphism generated by a certain primer (PIC), registered values between 0.29 and OPA-05 and 0. 49 to P-1. Dalkilic et al. (2011), obtained values that ranged from 0.16 to 0.50 in male fig (*Ficus carica caprificus* L.) genotypes using a RAPD marker, whereas this value was recorded to be 0.79 and 0.94 in fig (*Ficus Carica L.*) using AFLP and SSR markers, respectively. The discrimination index (PI) showed values between 1.82 for the OPA-10 primer and 4.04 for the OPA-11 primer. This primer had the highest capacity to generate polymorphic bands in the fig varieties studied.

Table 2. Polymorphism rate of fig cultivars through RAPD primers

Primers	Sequence	RAPD total bands	monomorphic fragments	polimorphyc fragments	% polimorphyc marker	PIC		pi
OPA05	AGGGGTCTTG	8	0	8	100	0.29	0.03	2.37
OPA 10	GTGATCGCAG	6	1	5	83.33	0.36	0.08	1.82
OPA11	CAATCGCCGT	10	1	9	90	0.44	0.04	4.04
OPG 02	GGCACTGAGG	11	2	9	81.81	0.37	0.04	3.35
P1	ACACAGAGGG	7	1	6	85.75	0.49	0.07	2.96
OPT20	GACCAATGCC	9	1	8	88.88	0.38	0.05	3.06
average		**8.5**	**1**	**7.5**	**91.916**			
total		**51**	**6**	**45**				

On the basis of genetic similarity among genotypes, the corresponding dendrogram was prepared by the UPGMA method. The analyzed cultivars were divided into two clusters.



The first cluster consists of the Timisoara cultivar and most of the Svinita cultivars, showing more genetic similarity among each other. Cultivar Svinita 1 is similar to Svinita 4 at a rate of 72%. Sebis cultivars clustered in the second group, which is similar to Svinita 6 at a rate of 60%. An appropriate genetic variability in hybrid populations can be obtained using cultivars from different clusters and subclusters. The similarity matrix obtained using the Jaccard coefficient ranged from 0.490 to 0.725 in the cultivars tested in this study. The varieties tested in this study showed a large divergence in DNA levels.

• Conclusions

- We conclude that there is important genetic diversity at the DNA level among the fig cultivars from the western region of Romania. The similarity coefficient ranged from 0.4902 to 0.725 for the cultivars tested.
- Using RAPD could be considered an effective tool for detecting phylogenetic relationships and similarity of study cultivars. The RAPD analysis can be considered an efficient technique for fingerprinting Romanian fig cultivars, and the results obtained could be incorporated into breeding programs.
- The use of molecular markers in plant characterization is an ideal approach for improving and conserving plant genetic resources.