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Full Length Research Paper

Identification and differentiation of *Ficus carica* L. cultivars using inter simple sequence repeat markers

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Information on germplasm diversity and relationships among elite materials is fundamentally important in crop improvement. The main objectives of our study is to determine the level of genetic diversity inter fig-tree cultivars using inter simple sequence repeat (ISSR) markers. Fifty-seven local Tunisian fig-tree cultivars were fingerprinted with ISSR marker. A total of 33 alleles were detected. A high level of genetic diversity was identified inter cultivars. The clustering grouped the studied cultivars into four clusters with no correlation to geographical origins.

Key words: *Ficus carica* L., genetic diversity, ISSR markers, Southeast Tunisia.

INTRODUCTION

Fig-tree (*Ficus carica* L.) is a crop of major importance in Tunisia, and both the area planted (30.000 ha) and yield (35.000 tons) has dramatically increased during the last decade. This diploid species (2n=26) of the family of *Moraceae*, is well adapted to bioclimatic conditions of Mediterranean basin.

The fig is very nourishing food and used in industrial product (Condit, 1947). It is very energizing, rich in vitamin, mineral elements, water and fat matter. Identification and characterization of fig-tree cultivars in Tunisia have started by Minangoin (1931) who have described some local cultivars. Other prospections in central Tunisia revealed the presence of a high diversity (Lahbib, 1984).

In South Tunisia (Beni Kheddache, Tataouine, etc), some clones were evaluated for agronomic performance (Ancillotti, 1988; Aljane, 2004). These works have revealed the presence of several clones with different morphological, agronomic and ecological characteristics.

Apart from morphological, physiological and agronomic traits (all of them being phenotypic), the genetic analysis by molecular markers assessment is very important for cultivars and clones characterisation. Several reasons contribute to that, especially the restricted number and low heritability of phenotypic characters; the difficulty in obtaining an accurate distinction between different cultivars before plants has attained the adult phase of life etc.

In addition, molecular markers not only provide a useful method for cultivars characterisation, but they also allow genetic relatedness among cultivars and clones to be assessed and determined more accurately.

During the last decade several novel DNA-markers (RAPD, RFLP, SSR, ISSR etc.) have emerged and have been rapidly integrated into the tools available for genome analysis. The ISSR, based on the amplification of regions (100-3000 bp) between inversely oriented closely spaced microsatellites (Salimath et al., 1995) has been used for DNA fingerprinting and assessing genetic diversity (Martin and Sanchez–Yelamo, 2000), principally of cultivated plants (Kantety et al., 1995; Charters et al., 1996; Nagaoka and Ogihara, 1997; Moreno et al., 1998; Blair et al., 1999; Fernandez et al., 2002). The production of large numbers of fragments, the reproducibility, and the low costs are advantages in the use of ISSR markers (Salimath et al., 1995; Moreno et al., 1998).

In figs, the use of these techniques has been limited to a few studies using biochemical markers, such as isozymes (Cabrita et al., 2001). However, isozymes are less useful in classifying material because of the small number of available marker loci providing limited coverage of the genome. Hence, we become interested in the exploration of the Tunisian fig diversity at DNA level by

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Table 1. Fig tree cultivars studied with their origin (+: cultivars no studed).

	Nom	origin
1	Tayouri akdar	Beni Kdech- Zammour
2	Hamouri	=
3	Safouri	=
4	Sawoudi	=
5	Bayoudi	=
6	Khadouri	Tataouine-Chnenni
7	Rogabi	=
8	Makbech	=
9	Hami	=
10	Magouli	=
11	Zidi	=
12	Magouli akhel	Tataouine-Edwiret
13	Hami	
14	Bayoudi	=
15	Sawoudi	=
16	Wedlani	=
17	Bither	=
18	Sawoudi bdar	=
19	Ragoubi kchine	=
20	Ragoubi	=
21	Makbech	=
22	Tayouri jwayed	=
23	+	+
24	Bayoudi	=
25	Makbech sawoudi	=
26	Magouli ekchine	=
27	Thokar bou harrak	=
28	Bither thokari	=
29	Romani	=
30	Minouri	=
31		
32	Makbech	Zarzis
33	Jbeli	Zarzis
34	Soltani	Zarzis
35	Zidi	Mesjed aissa
36	kahli	Kalaa kebira
37	Ragoubi	Beni kheddache
38	Wedlani	Beni kheddache
39	Bither	Zarzis
40	+	+
41	Bither	Maghni
42	Besbassi	Zammour
43	Chetoui	Zammour
44	Magouli	Ras el oued
45	Tayouri ahmer	Ras el oued
46	Romani	Bir amir
47	Bayoudi	Maghni
48	Bither	Ras el oued
49	Marsa matrouh	Ras el oued
50	Felyoui	Ras el oued
51	Minouri	Dwiret
52	Safouri	Beni kheddache
53	Sawoudi	Ras el oued
54	Tayouri asfer	Ras el oued
55	hamouri	Nekrif
56	Croussi	Beni kheddache
57	Jemaâoui	Zammour

the Inter Simple Sequence Repeat (ISSR) maker. This technology has been used to DNA fingerprint a wide range of crops. The objectives of the present study were to determine the levels of genetic diversity inter fig-tree cultivars in South Tunisia.

MATERIAL AND METHODS

Plant material

This study was performed using a 57 local Tunisian fig-trees cultivars (Table 1). The plant material consisted of young leaves that were sampled from adult trees to the fig germplasm collections maintained at IRA (Institut des Régions Arides, Medenine).

DNA extraction

Genomic DNA was extracted from fresh leaves of single adult trees following the method described by Doyle and Doyle (1987) with minor modifications. DNA concentration was determined by both spectrophotometry at 260 nm and 0.8% agarose gel electrophoresis.

ISSR PCR amplification and electrophoresis

DNA samples of the 54 individuals plants were adjusted to 20 ng/µl and used in the amplification reactions with a final volume of 25 µl containing 1 µl of DNA, 2 µl of primer (40 µM), 1 µl of dNTPs (10 mM), 0.2 µl Taq DNA polymerase (5 U/µl), 3 µl PCR buffer, 1.5 µl of MgCl2 (25 mM) and 16.3 µl dionized water.

DNA amplification was carried out using a Techne (Genius) thermal cycler programmed with 3 min at 94°C for initial denaturation, followed by 35 cycles of 54s at 94°C, 45 sec at 43°C, 2 min at 72°C, and a final 5 min extension at 72°C. After amplification, the DNA fragments were separated by electrophoresis for about 2 h under constant voltage (60 V) in 2% agarose gel submersed in 1X TBE buffer. The gels were stained with ethidium bromide solution and observed under ultraviolet light. Each gel was photodocumented using the image capturing system bioprint. The Jules DNA ladder (QBiogene) was used as standard molecular weight marker.

Statistical analysis

The amplified bands were scored as 1 and 0 based on band (allele) presence and absence, respectively. Sizes of amplified bands were estimated using Gel Pro analyzer software. The similarity of all samples for all scored bands was assessed using Dice's similarity coefficient. The matrice generated were analyzed with SPSS version 12 software to evaluate genetic distance.

RESULTS

Molecular Polymorphism

The DNA analysis resulted in 33 bands. Across all cultivars studied, these 33 bands are polymorphic. Figure 1 shows the DNA fragments obtained with primer $(CA)_{6GG}$ Examining each cultivar, we can deduct that:

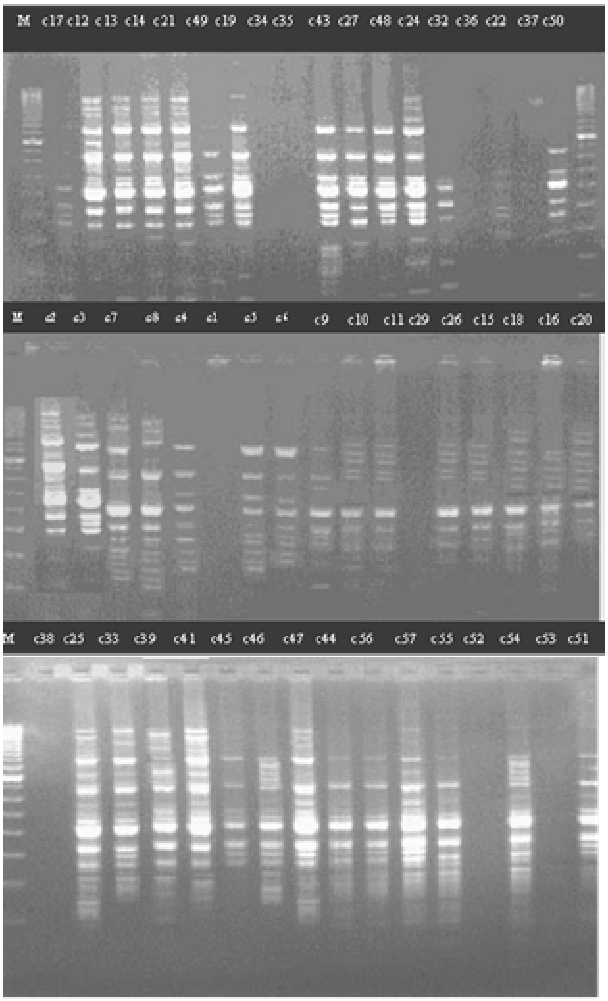

Figure 1. Electrophoresis pattern obtained in the ISSR primer $(CA)_{6GG}$ in 57 cultivars of fig trees.(c1…..c54 = cultivars, M is 100 bp DNA ladder).

1. The cultivars Makbech (Chnenni), Bayoudi (Edwiret), Makbech Sawoudi (Edwiret), Rogabi (Chnenni) and Felyoui (Ras el Oued) are characterized by an elevated polymorphous locus percentage (between 40% and 48%);
2. The cultivars Bither (Edwiret) and Tayouri jwayed (Edwiret) are characterized by a weak polymorphism percentage (15%);
3. The other cultivars present a moderate polymorphism (between 25% and 35%). The variation of the polymorphism in the different cultivars can be explained by the two following hypothesis:

The microsatellites whose sequences are complementtary to the primer, are abundant or rare in the genome of the studied cultivar, these microsatellites occupy some sites sufficiently distant not allowing the synthesis of sequences that separates them.

Relationships among cultivars of fig-tree

Cluster analysis (Figure 2) divided the 57 cultivars into four large groups:

1. Group 1 formed by the cultivars Bither (Edwiret), Tayouri jwayed (Edwiret), Makbech (Edwiret) and Felyoui (Ras el oued) that can be considered genetically very distant of the other cultivars. The cultivars revealed a very weak polymorphic percentage (15.15%);
1. Group 2 regroups about 20 cultivars characterizing by an elevated polymorphic percentage. The bands exhibit a molecular mass in the range of 600-1200 bp;
2. Group 3 contained about 20 cultivars presenting a moderate polymorphic percentage;
3. Group 4 formed by only one cultivar Sawoudi (Edwiret).

DISCUSSION AND CONCLUSION

The method described by Doyle and Doyle (1987) with some modifications proves to be more appropriate for the fast obtaining a good quality of DNA of fig tree for the amplification by PCR. The utilization of microsatellites markers in order to study diversity of different cultivars of fig-tree revel the presence of 33 fragments.

This study provides evidence that the ISSR procedure is an informative and suitable approach to the examination of the molecular polymorphism and the phylogenic relationships in the fig germplasm. This ISSRs technique have been used with success for the characterization of genetic polymorphism for the maize (Kantety et al., 1995), rice (Blair et al., 1999), potato (Mcgregor et al., 2000), Olive-tree (Terzopoulos, 2005), *Microsporum canis* (Jose, 2005) etc. Lately, this technique has been used to study the genetic relations between the different species of coffee and to determine the relationship between hybrids (Paulo, 2003). In the same way this technique proved to be efficient for the survey of the genetic variation at Apiaceae smyrnioides (Ying-Xiong et al., 2004).

The genetic diversity and the phylogenetic relation between 17 ecotypes of the fig-tree installed in collection at Chott Mariam (North Tunisia) is recently analyzed by Chatti et al. (2003) using the technique of RAPD. This study showed a significant morphological variation and a

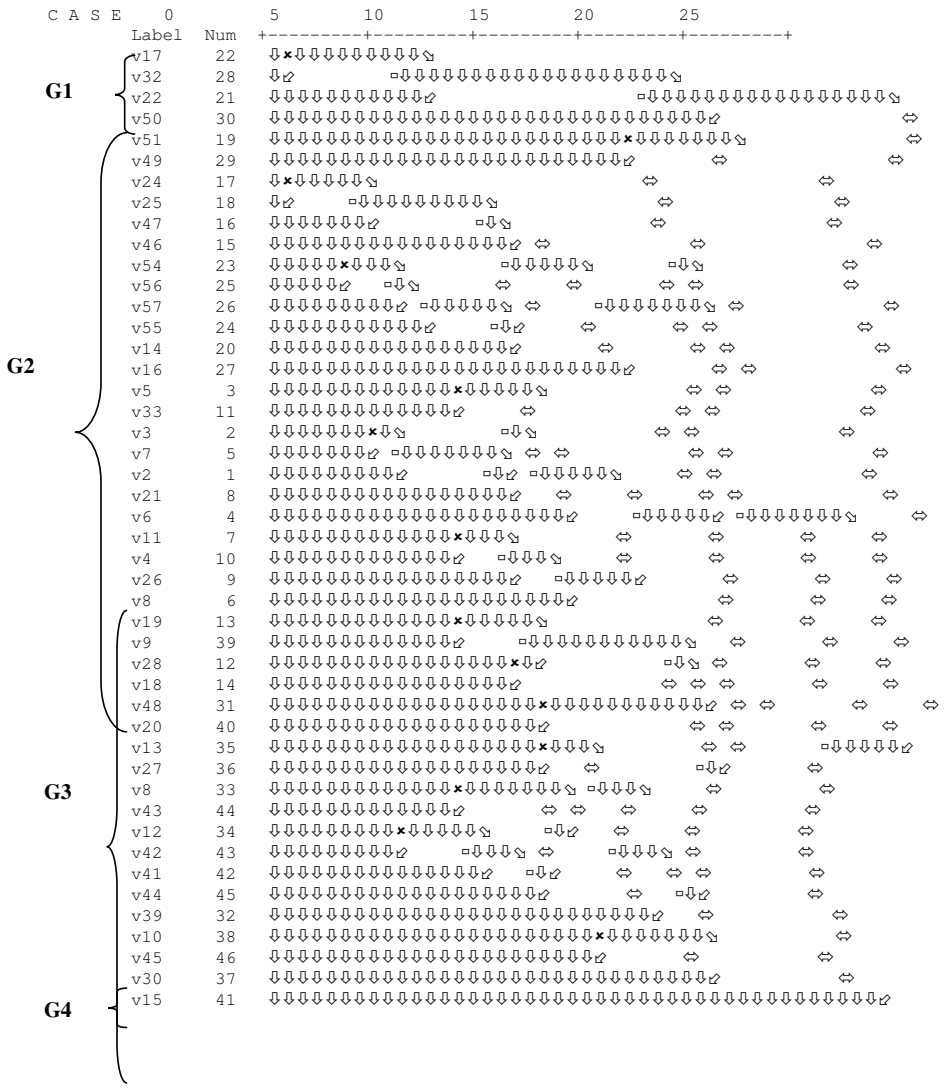

Figure 2. Dendrogram of 57 fig trees cultivars based in Dice similarity index.

large genetic diversity within and among cultivars. It is acknowledged that there is a strong diversity within and among cultivars of fig tree in Tunisia and this phenomenon has led to the success to the improvement in crop quality and yield.

The overall results of this investigation demonstrated that all genotypes studied could provide valuable material for use in breeding programs. Finally more works are necessary to enlarge the number of markers by the use of other molecular technologies in order to have a deeper insight into the molecular polymorphisms and to establish a varietal identification key in this crop.

REFERENCES

Aljane F (2004). Prospection and characterization of the local varieties of fig-tree (*Ficus. carica* L.) In: Jebels Matmatas. Master of Sciences. University of Sfax, Sfax,Tunisia.

Blair MW, Panaud O, Mc Couch SR (1999). Inter- simple sequence repeat (ISSR) amplification for analysis of microsatellite motif frequency and fingerprinting in rice (Oriza sativa L.). Theor. Appl. Genet. 98: 780-792.

Cabrita Luis F, Aksoy U, Hepaksoy S, Leitão Jose M (2001). Suitability of isozyme, RAPD and AFLP markers to assess genetic differences and relatedness among fig (*Ficus carica* L.) clones. Scientia Horticulturae. 87: 261-273

Charters YM, Robertson A, Wilkinson M J (1996). PCR analysis of oil seed rape cultivars (Brassica napus L.ssp. ollifera) using 5'-anchored simple sequence repeat (SSR) primers.- Thor. Appl. Genet. 92: 442-447.

Chatti K, Salhi- Hannachi A, Mars M, Marrakchi M, Trifi M (2003). Genetic Diversity and phlogenic relationships in Tunisian fig (Ficus carica L.) cultivars mediated by RAPD. Biology. 2: 1- 4.

Condit IJ (1947). Fig tree. Erans Verdoorn, USA, pp. 1- 187 ..

Doyle and Doyle (1987). A rapid DNA isolation procedure for small quantities of fresh leaf tissues. Phytoch. Bull. 19: 11-15.

Fernandez ME, Figueiras AM, Benito C (2002). The use of ISSR and RAPD markers for detecting DNA polymorphism, genotype identification and genetic diversity with known origin. Thor. Appl. Genet. 104: 845–851.

Jose C, Antonio R, Marıa S, Joaquina G, Marıa C, Rubio MJ, Revillo JG (2005).Inter-single-sequence-repeat-PCR typingas a new tool for identification of Microsporum canis strains. Journal of Dermatological Science. 39: 17-21.

Kantety RV, Zeng XP, Bebbetzen JL (1995). Assessment of genetic diversity in dent and popcorn (*Zea mays* L.) inbreed lines using inter-simple sequence repeat (ISSR) amplification. Mol. Breeding. 1: 365-373.

Lahbib T (1984). Morphological study of the varieties of fig tree (*Ficus carica* L.) cultivated in the Tunisian Sahel. Master of Sciences, University of Tunis, Tunis, Tunisia.

Martin JP, Sanchez–Yelamo MD (2002). Genetic relationships among species of the genus Diplotaxis (Brassicaceae) using inter simple sequence repeat markers. Theor. Appl. Genet. 101: 1234–1241.

Mc Gregor CE, Lambert CA, Greyling MM (2000). Comparative assessment of DNA fingerprinting techniques (RAPD, ISSR, AFLP and ISSR) in tetraploid potato *(Solanum tuberosum* L.). Euphytica, 35: 13-44.

Minangoin N (1931). Monography of the Tunisian varieties of fig tree. In: Baconnier (edit) Proceeding of Agronomic Congress held at Algiers, Algeria, pp. 336-364.

Moreno S, Martin JP, Ortiz JM (1998). Inter simple sequence repeat PCR or characterization of closely related grapevine germplasm. Euphytica. 101: 117–125.

Nagaoka T, Ogihara Y (1997). Applicability of inter simple sequence repeat polymorphism in wheat for use as DNA markers in comparison to RFLP and RAPD markers. Theor. Appl. Genetic. 94: 597–602.

Terzopoulos PJ, Kolano B, Bebelia PJ, Kaltsikes PJ, Metzidakis I (2005). Identification of Olea europaea L. cultivars using inter-simple sequence repeat markers. Scientia Horticultura. 105: 45–51.

Paulo M, Ruas Claudete F, Ruas Leandro R, Valdemar P, Carvalho Eduardo A, Tumoro S (2003). Genetic relationship in coffea species and parentage determination of interspecific hybrids using ISSR (Inter- Simple Séquence Repeat). Genet. Mol. Bio. 26: 10-12.

Salhi-Hannachi A, Chatti K, Mars M, Marrakchi M, Trifi M (2003). Comparative analysis of genetic diversity in two tunisian collections of fig cultivars based on random amplified polymorphic DNA and inter simple sequence repeats fingerprints. Genetic Resources and Crop Evolution, 13: 1-11.

Salimah SS, De Oliveira AC, Godwin ID (1995). Assessment of genomic origin and genetic diversity in the genus Eleusine with DNA markers. Genomic. 38: 757–763.

Ying-Xiong Q, De Yuan H, Cheng XF (2004). Genetic variation in the endangered and endemic species *Changium smyrnioides* (*Apiaceae*). Biochemical Systematic and Ecology. 32: 583-596.