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Comparative Assessment of SSR and AFLP Markers for Evaluation of Genetic Diversity and Conservation of Fig, *Ficus carica* L., Genetic Resources in Tunisia

Ghada Baraket · Khaled Chatti · Olfa Saddoud · Ahmed Ben Abdelkarim · Messaoud Mars · Mokhtar Trifi · Amel Salhi Hannachi

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Abstract This study characterises the genetic variability of fig, *Ficus carica* L., using simple sequence repeat (SSR) and amplified fragment length polymorphism (AFLP) markers. It compares the efficiency and utility of the two techniques in detecting variation and establishing genetic relationships among Tunisian fig cultivars. Our results show that using both marker systems, the Tunisian fig germ plasm is characterised by having a large genetic diversity at the deoxyribonucleic acid level, as most of AFLP bands were detected and all SSR markers were polymorphic. In fact, 351 (342 polymorphic) and 57 (57 polymorphic) bands were detected using AFLP and SSR primers, respectively. SSR markers were the most polymorphic with an average polymorphic information content value of 0.94, while AFLP markers showed the highest effective multiplex ratio (56.9) and marker index (45.2). The effective marker index was recorded highest (4.19) for AFLP markers and lowest (0.70) for the SSR ones. Our results demonstrate that (1) independent as well as combined analyses of cluster analyses of SSR and AFLP fragments showed that cultivars are clustered independently from their geographical origin, horticultural classifications and tree sex; (2) the analysis of molecular variance allowed the partitioning of genetic variation within and among fig groups and showed greater variation within groups and (3) AFLP and SSR markers datasets showed positive correlation. This study suggests the SSR and AFLP markers are suitable for diversity analysis and cultivars fingerprinting. An understanding of the genetic diversity and population structure of *F. carica* in Tunisia can also provide insight into the conservation and management of this species.

Keywords AFLP · Genetic diversity · Fig, *Ficus carica* L. · SSR · Tunisia

Abbreviations

SSR	Simple sequence repeat
AFLP	Amplified fragment length polymorphism
RAPD	Random amplified polymorphic DNA
ISSR	Inter simple sequence repeats
RAMPO	Randomly amplified microsatellite polymorphism
RFLP	Restriction fragment length polymorphism
ITS	Internal transcribed spacer
E	Effective multiplex ratio
EMI	Effective marker index
MI	Marker index
n	Multiplex ratio
QND	Qualitative nature of data
DC	Documentation capability
QM	Quality of marker
PR	Percent reproducibility
PIC	Polymorphic information content
DNA	Deoxyribonucleic acid
PCR	Polymerase chain reaction
UPGMA	Unweighted pair group method with the arithmetic averaging algorithm

G. Baraket · K. Chatti · O. Saddoud · A. B. Abdelkarim · M. Trifi · A. S. Hannachi (✉)
Laboratory of Molecular Genetics, Immunology and Biotechnology, Faculty of Sciences of Tunis, Campus University, 2092 El Manar,
Tunis, Tunisia
e-mail: Amel.SalhiHannachi@fsb.rnu.tn

M. Mars
U.R. Agro-biodiversity, Higher Agronomic Institute, 4042 Chott Mariem,
Sousse, Tunisia

PCA Principal component analysis
AMOVA Analysis of molecular variance

Introduction

Molecular markers provide novel tools to differentiate among different plant genetic resources. Different marker systems are currently available for monitoring genetic diversity. Model markers with contrasting characteristics include amplified fragment length polymorphism (AFLP) as dominant and biallelic markers (Vos et al. 1995) and simple sequence repeat (SSR) as co-dominant markers that usually reveal a high number of alleles (Tautz 1989). AFLP technique also provides many markers that randomly spread in the genome, whereas in a diversity study, the number of analysed microsatellites is usually low. The comparative analysis between different markers is therefore intended to reveal the balance between different evolutionary forces contributing to genetic diversity. Fig (*Ficus carica* L.) trees are one of the earliest cultivated fruit-bearing trees. *F. carica* is considered morphologically gynodioecious but functionally dioecious. According to reproductive organs, there are two main types of trees: the caprifig (male tree) and the female tree (Weiblen 2000). Today, the fig is a moderately important world crop, with an estimated annual fruit production of one million tonnes (Sadhu 1990). Tunisian fig biodiversity is very high, and many niches are well suited for fig production. Despite its socio-economic and historical importance, fig tree was considered as a secondary crop. In Tunisia, the area planted (30,000 ha) and yield (35,000 t) have dramatically increased during the last decade. Moreover, severe genetic erosion is also threatening the remaining germ plasm. Thus, information available on ancient landraces is very limited (Mars et al. 2008). In order to conserve local fig germ plasm, prospection and collection were undertaken since the late 1980s. Five ex situ collections were established throughout the country (Mars et al. 2008). In addition, description of Tunisian germ plasm was reported using pomological and agronomic characters. Morph metric studies showed considerable variability among Tunisian fig cultivars (Mars and Marrakchi 1998; Chatti et al. 2004a; Salhi Hannachi et al. 2003; Saddoud et al. 2008). Biochemical and molecular markers such as isozymes, random amplified polymorphic DNA (RAPD), inter simple sequence repeats (ISSR), randomly amplified microsatellite polymorphism (RAMPO), SSR, AFLP and internal transcribed spacer (ITS) (rDNA) have also been applied. Results of such studies provided evidence of a significant but unstructured genetic diversity (Hedfi et al. 2003; Chatti et al. 2004b; Salhi Hannachi et al. 2004, 2005, 2006; Baraket et al. 2009a, b).

As a part of our research, we applied the two deoxyribonucleic acid (DNA) markers, SSR and AFLP, for more comprehensive and precise estimation of the unknown genetic background, structure, relationships and diversity of fig cultivars distributed in Tunisia. Therefore, we have considered the practical issues of choosing marker systems in order to facilitate selection of the appropriate technology for a given aim; we have compared these two kinds of molecular markers using several parameters to know if both markers would give the same information for conservation purposes.

In fact, to classify the examined figs in different groups, the characteristic properties for all two types of marker assays including polymorphic information content (PIC), as a function of a marker system's ability to distinguish among genotypes; multiplex ratio (n), which defines the number of bands analysed simultaneously per experiment; marker index (MI), as a universal metric to represent the amount of information obtained per experiment for a given kind of markers as reported by Powell et al. (1996) and two new estimates termed qualitative nature of data (QND) and effective marker index (EMI) were discussed. Additionally, determination of genetic relationships between fig genotypes were established for the two marker assays (AFLP and SSR) and the pooled data and compared for congruency. Information can be used for rational design of breeding programs, conservation of local germ plasm and management of fig genetic resources.

Materials and Methods

Plant Material

Thirty-eight accessions (30 varieties and eight male trees) representing five regions from Tunisia were used in this study (Table 1). Plant material consisted of young leaves (approximately 10 g) sampled from adult trees.

DNA Isolation

Young leaves were sampled from the field. Genomic DNA was extracted as described by Dellaporta et al. (1983). The quality and quantity of the DNA were evaluated by analysis on a 0.8% agarose gel. DNA was diluted appropriately for polymerase chain reaction (PCR) assays.

SSR Analysis

Six SSR primers designed for fig were used in this study (MFC2, MFC3, MFC5, MFC6, MFC7 and MFC8; Khadari et al. 2001). All produced clear electrophoretic profiles, uncovering a high level of polymorphism in fig varieties. Amplification and detection were performed following

Table 1 List of 38 Tunisian fig cultivars studied and their geographical origin

Accession name	Label	Horticultural classifications	Geographical origin	
Soltani 1	SL1	*Uniferous*	Ourdanine	Sahel
Kahli 1	KH1	*Uniferous*	Kalaa Kebira	
Hemri 1	HM	*Uniferous*	Enfidha	
Zidi 1	ZD1	*Uniferous*	Mesjed Aissa	
Baghali	BG	*Uniferous*	Mesjed Aissa	
Bidhi	BD	*Uniferous*	Kalaa Kebira	
Bither abiadh	BA	*Biferous*	Mesjed Aissa	
Besbessi	BS	*Biferous*	Mesjed Aissa	
Jrani	JR	*Uniferous*	Ghadhabna	
Assafri	AS	*Uniferous*	Ghadhabna	
Zergui	ZG	*Uniferous*	Dégache	South West
Zidi 3	ZD3	*Uniferous*	Tozeur	
Hamri	HMR	*Uniferous*	Dégache	
Khadhri	KDR	*Uniferous*	Dégache	
Khartoumi	KR	*Uniferous*	Dégache	
Tounsi	TS	*Uniferous*	Dégache	
Wahchi	WH	*Biferous*	Dégache	
Chetoui1	CH1	*Biferous*	Dégache	
Dhokkar 2	DH2	*Uniferous*	Dégache	
Sawoudi	SD	*Uniferous*	Gafsa	
Gaa Zir	GZ	*Uniferous*	Gafsa	
Assal boudchiche	AB	*Uniferous*	Gafsa	
Khadhouri	KD	*Uniferous*	Gafsa	
Dhokkar 1	DH1	*Uniferous*	Gafsa	
Hammouri	HR	*Uniferous*	Medenine	South East
Widlani	WD	*Uniferous*	Medenine	
Zaghoubi	ZH	*Uniferous*	Medenine	
Rogabi	RG	*Uniferous*	Medenine	
Makhbech	MK	*Uniferous*	Medenine	
Dhokkar Zarzis	DZ	*Uniferous*	Medenine	
Zidi 2	ZD2	*Uniferous*	Utique	North East
Dhokkar 4	DH4	*Uniferous*	Utique	
Dhokkar 5	DH5	*Uniferous*	Raf Raf	
Chetoui2	CH2	*Biferous*	Raf Raf	
Temri	TM	*Uniferous*	Kerkenah	Kerkenah
Baghli	BGL	*Uniferous*	Kerkenah	
Abiadh	ABD	*Uniferous*	Kerkenah	
Dhokkar 3	DH3	*Uniferous*	Kerkenah	

Saddoud et al. (2005). The amplified banding patterns were firstly checked on 2% agarose gels visualised with ethidium bromide staining under UV light. SSRs were resolved on non-denaturing polyacrylamide gels (10%) and visualised by ethidium bromide staining as described by Sambrook et al. (1989). Alleles were scored according to molecular weight markers.

AFLP Analysis

The protocol was adapted from Vos et al. (1995), as described by Baraket et al. (2009b). Initially, eight primer combinations were tested against 38 individuals from five cultivar groups. All primer pairs generated profiles with a high proportion of polymorphic bands. From this pilot study, the six primer combinations, E_{AAC}/M_{CTA}, E_{ACC}/M_{CTA}, E_{ACA}/M_{CAG}, E_{AAC}/M_{CAT}, E_{AAC}/M_{CAG} and E_{AGC}/M_{CAA}, were selected. A reproducibility test was performed on 37 plants with all six primer combinations. For each sample, AFLP fingerprints were compared. Amplified fragments were separated on denaturing gels (6%) and silver stained. Each PCR product (60 to 650 bp) was assumed to represent a single locus with two alleles; data were scored as the presence (1) or absence (0) of each polymorphic band.

Data Analysis

For each primers combination (SSR and AFLP oligonucleotides), the total number of bands was determined, and only the polymorphic ones were taken into account to estimate the percentage of polymorphic bands (PPB). PIC, effective multiplex ratio (E), MI, QND and EMI were calculated as reported (Lynch and Walsh 1998): $\mathrm{PIC} = 1 - \sum_{i=1}^{k} Pi^2$, where k is the total number of alleles detected for a given marker locus, and Pi is the frequency of the ith allele in the set of genotypes investigated. $E = n \times \beta$, where β is the fraction of polymorphic markers, and it is estimated after considering the polymorphic loci (n_p) and non-polymorphic loci (n_{np}) as $\beta = n_p/(n_p + n_{np})$. The multiplex ratio ($n$) is the average number of DNA fragments amplified/detected per genotype using a marker system. A product of information content, as measured by PIC, and effective multiplex ratio, called as MI, may provide a suitable estimate of marker utility (Powell et al. 1996): MI=PIC×E. The QND depends on many factors such as reproducibility and amenability of bands. For easy documentation (e.g. precise allele sizing and storing in databases), it is defined as QND=DC×QM×PR, where DC is the documentation capability, QM is the quality of marker and PR is the percent reproducibility of the fragment(s)/band(s) of the given marker system across the laboratories. DC and PR represent the constant value for a given marker type; however, QM is a feature of the primer pair for a marker type, and it shows a variable value. The constant values for DC and PR for different markers have been set as follows: DC value of SSR markers has been set at 0.75 and 0.25 of AFLP markers because results of SSR are easier to record but allele sizes need to be measured. However, for AFLP results, it is very difficult to record or document as it is not possible to size all the AFLP loci obtained by a primer pair until the AFLP experiments are conducted on ABI machines. While conducting AFLP experiments on ABI machines, the DC value for AFLP can be used as 0.50 or between 0.50 and 0.75 (Varshney et al. 2007). PR value for both SSR and AFLP markers is given 1 and 0.50, respectively, as results of these markers are most likely to be reproduced on different analysing systems and across the laboratories. The QM value, however, will differ with the primer pair even for a given marker type. Therefore, the user needs to define the QM value as per the experiments according to the following scale (Varshney et al. 2007): 1.00, good quality marker—single and strong band/peak; 0.75, faint band or lower peak; 0.50, marker/band with stuttering and 0.25, difficult to score (needs special efforts to visualise). Finally, the EMI, a possible measure to approximate the overall efficacy of a marker system taking into account all the parameters mentioned above, can be calculated as reported (Varshney et al. 2007): EMI=MI×QND.

Cluster Analysis

Banding profiles generated by SSRs and AFLPs were compiled into a data binary matrix based on the presence (1) or absence (0) of the selected band. Data were then computed with the Genedist program to produce a genetic distance matrix using the formula of Nei and Li (1979). Based on the unweighted pair group method with the arithmetic averaging algorithm (UPGMA), the Neighbour program was used by computing the genetic distance matrix to produce tree file (Sneath and Sokal 1973). This tree file was computed with the TreeView program to draw a phylogenic diagram. All these analyses were carried out using appropriate programs of the Felsenstein's PHYLIP software (Felsenstein 1995) and the TreeView software of Page (1996). In addition, principal component analysis (PCA) was performed by computing the data matrix with appropriate programs of the SAS software (SAS 1990). Also, individual data obtained with SSR and AFLP markers were combined to prepare identical cluster analysis held above.

Molecular variance (AMOVA) analysis was carried out on each marker system (SSR and AFLP) and on a combined (SSR&AFLP) dataset using WINAMOVA (version 1.55) program (Excoffier 1993). The AMOVA components were used as estimates of molecular diversity at the hierarchical level among and within group categories. We made grouping as follows: group 1 (Sahel), group 2 (South West), group 3 (South East), group 4 (North East) and group 5 (Kerkenah). The significance of P values was tested non-parametrically after 1,000 permutations. Finally, AFLP and SSR matrices were compared by computing Spearman's rank correlations using the statistical packages STATISTICA 6.0 (StatSoft Inc. 2001). Two-dimensional histograms present a graphical representation of the frequency distribution of genetic distances generated using the selected marker in which the columns are drawn over the class intervals and the heights of the columns are proportional to the class frequencies. In addition, two-dimensional scatterplots, which to be the graphical equivalent of the correlation matrix, are shown for each pair of markers; the linear regression line is also shown in each scatterplot. The scatterplot visualises a relation (correlation) between two variables: X and Y (e.g. genetic distances). Individual data points are represented in two-dimensional space, where axes represent the variables (X on the horizontal axis and Y on the vertical axis). The two coordinates (X and Y) that determine the location of each point correspond to its specific values on the two variables. Correlation is a measure of the relation between two or more variables. Spearman rank parameter is a type of

Table 2 Summary of simple sequence repeat and amplified fragment length polymorphism primer combination (PC) characteristics

PC	TNB	NPB	PPB	PIC	Allele size (bp)
AFLP					
E_{AAC}/M_{CTA}	72	67	93.05	0.71	
E_{ACC}/M_{CTA}	61	59	96.72	0.89	
E_{ACA}/M_{CAG}	42	40	95.23	0.87	
E_{AAC}/M_{CAT}	66	66	100	0.79	
E_{AAC}/M_{CAG}	53	53	100	0.86	
E_{AGC}/M_{CAA}	57	57	100	0.65	
Total	351	342	–	–	
Average	58.5	57	97.5	0.79	
SSR					
MFC2	8	8	100	0.93	170–228
MFC3	12	12	100	0.98	124–176
MFC5	10	10	100	0.94	118–165
MFC6	7	7	100	0.90	166–188
MFC7	6	6	100	0.91	130–148
MFC8	14	14	100	0.98	141–202
Total	57	57	–	–	–
Average	9.5	9.5	100	0.94	

E_{NNN}/M_{NNN}, primer pairs, *E* and *M* for *Eco* RI and *Mse*I, respectively
TNB total number of bands, *NPB* number of polymorphic bands, *PPB* percentage of polymorphic bands, *PIC* polymorphic information content

commonly used non-parametric correlation coefficient (Siegel and Castellan 1988). The probability test *P* is used to test the usual null hypothesis (no difference between the two treatments) and to have sufficient evidence to allow rejection of the null hypothesis at a pre-determined alpha set of 0.05.

Results and Discussion

AFLP Analysis

Six primer pairs were tested for their ability to generate AFLP banding patterns from DNA corresponding to the 38 considered cultivars. A total of 342 polymorphic bands, ranging in size from 60 to 650 bp, were scored using the designated primer combinations (Table 2). The number of AFLP markers varied from 40 for E_{ACA}/M_{CAG} to 67 for E_{AAC}/M_{CTA}, with a mean of 57 bands per primer pair. Thus, the primers tested are useful to reveal DNA polymorphisms in fig, a conclusion supported by the high PPB scored using each primer combinations. Different levels of polymorphisms were detected since the PPB ranged from 93.05% for E_{AAC}/M_{CTA} to 100% for E_{AAC}/M_{CAT}, E_{AAC}/M_{CAG} and E_{AGC}/M_{CAA} primer combinations (Table 2). This result was significantly higher than that reported in Turkish fig by Cabrita et al. (2001). Using eight combinations of *Eco*RI/*Mse*I primers, 28% polymorphic bands were generated within fig clones studied. This result suggests that Tunisian fig germ plasm is characterised by having a large genetic diversity at the DNA level. In addition, The PIC values for individual primer combinations were recorded; however, the overall PIC values for individual primer combinations were in the range of 0.65 (E_{AGC}/M_{CAA}) to 0.89 (E_{ACC}/M_{CTA}) with an average of 0.79 per primer combinations (Table 2).

SSR Analysis

Diversity evaluation of the six SSR loci detected 57 alleles across the 38 individuals. The number of alleles per locus ranged from six (MFC7) to 14 (MFC8) with a mean of 9.5 (Table 2). The PPB is 100% for each SSR pair of primers. Thus, this marker type seems to be the most informative for assessment of diversity in fig and for discrimination among genotypes. The PIC values for these markers in the examined genotypes ranged from 0.90 (MFC6) to 0.98 (MFC3 and MFC8) with an average of 0.94. Similar results were reported by Ikegami et al. (2008) in European and Asian fig varieties. In the study herein, SSR was found highly polymorphic compared with AFLP markers. The high level of polymorphism linked with SSR is to be

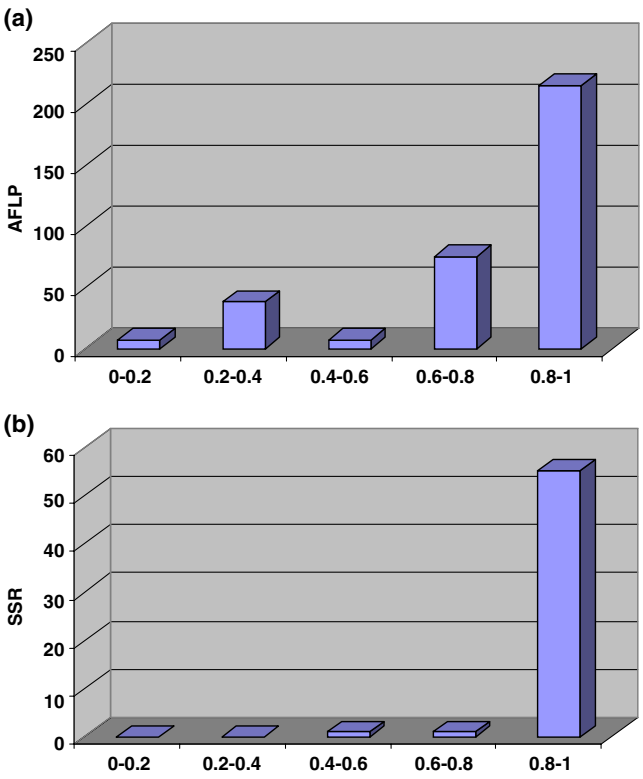

Fig. 1 Distribution of the polymorphism information content data obtained using (**a**) amplified fragment length polymorphism (AFLP) and (**b**) simple sequence repeat (SSR)

Table 3 Comparison of amplified fragment length polymorphism and simple sequence repeat marker systems

Marker system	No. of primer pairs analysed	No. of genetic loci amplified	Average (PIC)	Fraction of polymorphic markers (β)	Multiplex ratio (n)	Effective multiplex ratio, $E=n\times\beta$	Marker index, MI=$E\times$PIC	Qualitative nature of data (QND)	Effective marker index (EMI)
AFLP	6[a]	342 (351[b])	0.79	0.97	58.5	56.9	45.2	0.093	4.19
SSR	6	6	0.94	1	1	1	0. 94	0.75	0.70

[a] Primer combinations

[b] Total number of genetic loci is 351; however, only 342 polymorphic loci were taken in consideration

expected due to the unique mechanism responsible for generating SSR allelic diversity by replication slippage (Varshney et al. 2005; Gupta and Varshney 2000), whereas the basis of AFLP polymorphism are single nucleotide mutations and insertions/deletions (Ridout and Donini 1999; Rafalski 2002). Regarding the detection of polymorphism, SSR markers are better than that of AFLP as SSR are co-dominant and multiallelic markers in contrast to the nature of AFLP markers.

Comparative Utility of Marker Systems

Polymorphic Information Content (PIC)

SSR and AFLP markers showed very high level of polymorphism in the examined germ plasm as the PIC value for the SSR and AFLP markers was calculated with an average of 0.94 and 0.79, respectively, across the germ plasm lines assayed (Table 2). On the other hand,

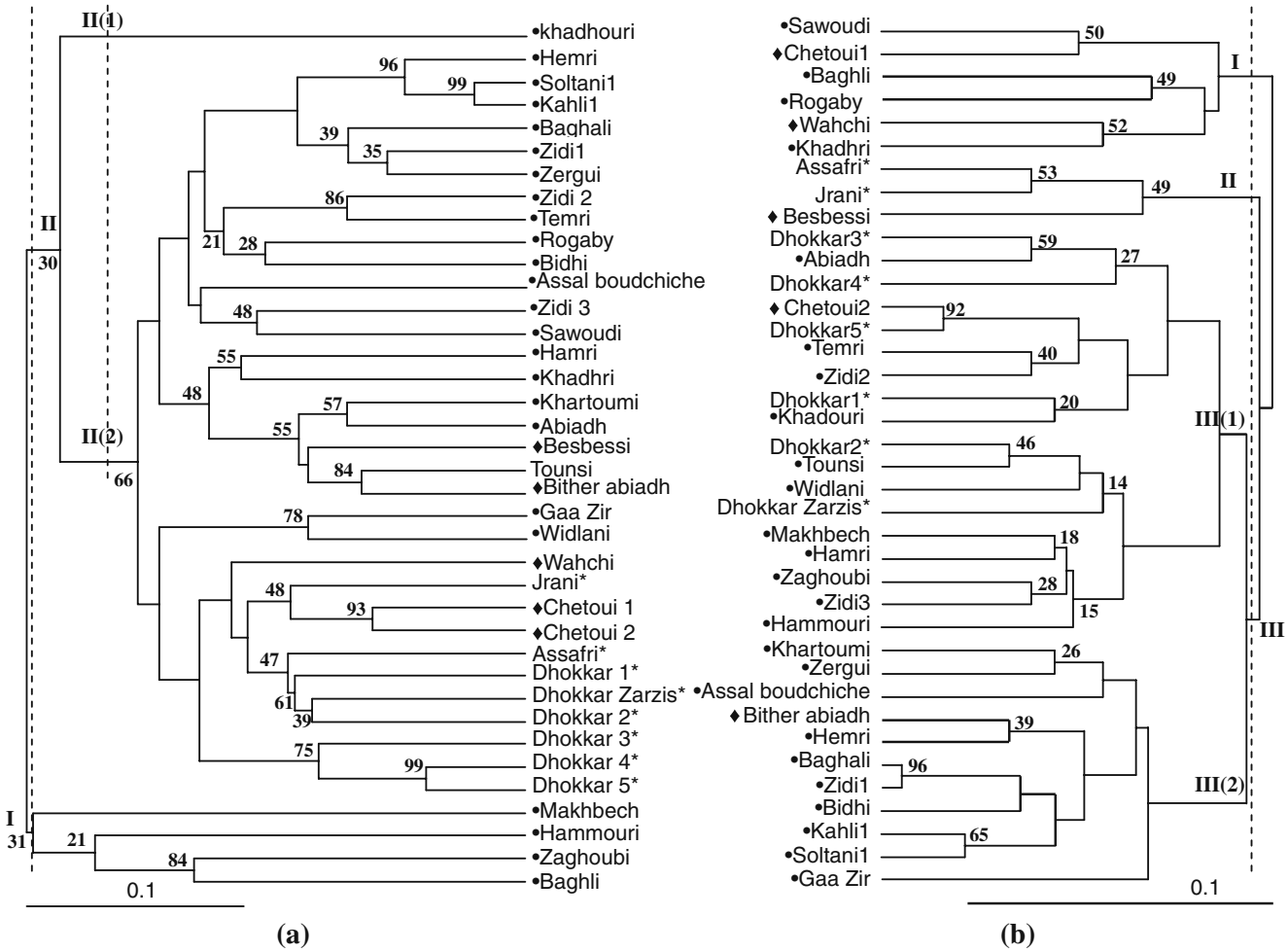

Fig. 2 UPGMA phenogram of the genetic relationships among 38 Tunisian fig accessions constructed from estimated Nei and Li (1979) genetic distance based on (**a**) 342 amplified fragment length polymorphism markers and (**b**) 57 simple sequence repeat (SSR) markers. The scale indicates the genetic distance. *Asterisks*, male trees; *circles*, Unifere cultivars; *diamonds*, Bifere cultivars

215 over the 342 AFLPs and 55 over the 57 SSRs exhibited PIC values ranging from 0.8 to 1. Therefore, the data proved that these markers are an attractive and powerful procedure to survey the genetic diversity of fig cultivars (Fig. 1a, b).

Multiplex (n) and Effective Multiplex Ratio (E)

Since SSR markers are usually locus specific, the six SSR markers analysed are considered equal to six loci (as one locus per primer pair) in the present study. Thus, the SSR markers also provided the multiplex or effective multiplex ratio as 1.0 per marker (Table 3). As a large number of fragments are detected in one gel lane, by using one AFLP primer combination, the AFLP markers have a higher multiplex ratio. Effective multiplex ratio of the AFLP, however, depends on the fraction of polymorphic markers (β) as many of the fragments obtained by one primer combination are polymorphic across the examined genotypes. In the present study, the n and E for the AFLP markers were calculated as 58.5 and 56.9, respectively (Table 3).

Marker Index (MI)

For determining the overall utility of a given marker system, the MI was calculated for all the two marker systems examined. The AFLP markers showed the highest MI (45.2), which is significantly higher than that estimated for the SSRs (0.94; Table 3). This analysis highlights the distinctive nature of the AFLP assay.

Qualitative Nature of Data (QND)

Another significant issue for different marker systems, we propose here, is the QND, produced by a marker. Based on the assumptions and weight ages mentioned under 'Materials and Methods', the QND for SSR markers was estimated at 0.75 as the highest and 0.093 as the lowest for the AFLP markers (Table 3).

Effective Marker Index (EMI)

The EMI considers all possible attributes such as information content, fraction of polymorphic fragments, multiplex ratio as well as the qualitative issues for a given marker system. According to our calculations, the EMI was at its highest (4.19) for the AFLP markers and lowest (0.70) for the SSR markers (Table 3). The effective multiplex ratio (E) for SSR was recorded as 1.0 because of assaying one genetic locus per primer pair. The MI, which is considered to be an overall measure of the efficiency to detect polymorphism, was highest (45.2) for AFLP and lowest

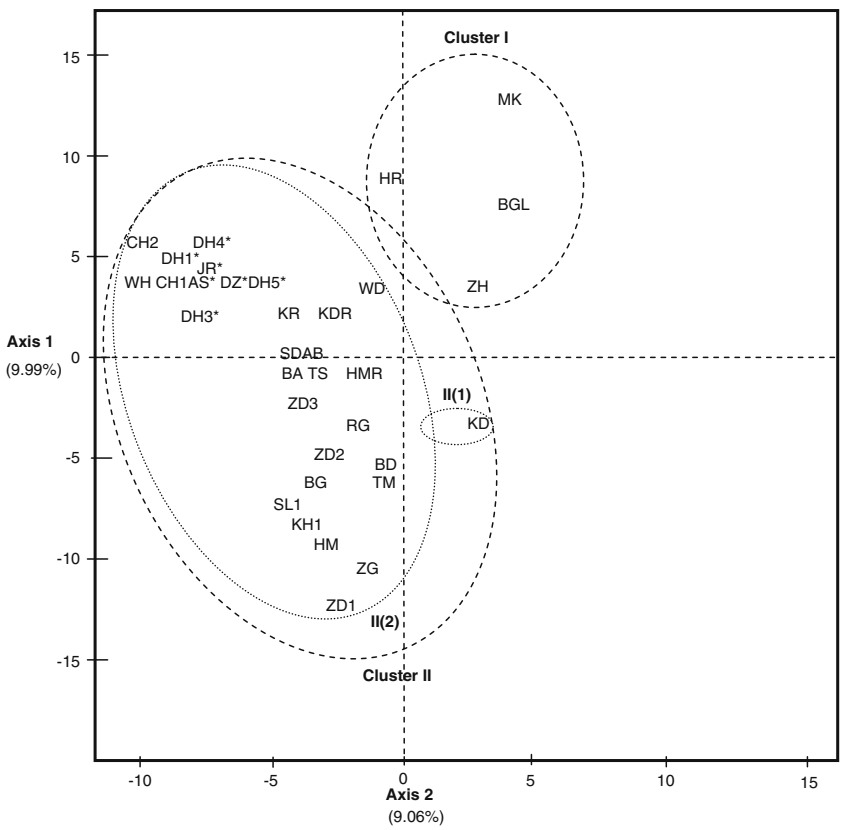

Fig. 3 Plot of 38 Tunisian fig genotypes according to the two first axes of the principal component analysis (PCA; 19.05% of the total genetic variability) based on 342 amplified fragment length polymorphisms (Table 1 for cultivar codes; *asterisks*, male trees)

Table 4 Relative contribution of each variable to the variation provided by the first three axis of the principal component analysis

Component	AFLP			SSR			AFLP and SSR		
	Axis 1	Axis 2	Axis 3	Axis 1	Axis 2	Axis 3	Axis 1	Axis 2	Axis 3
% Variation	9.99	9.06	7.48	10.48	8.98	8.17	9.08	8.50	6.89
% Cumulated	9.99	19.06	26.55	10.48	19.46	27.64	9.08	17.58	24.47
Variable contributing to the definition of the PCA axis	$E_{AAC}/M_{CTA(63)}$ (+0.114)	$E_{AAC}/M_{CTA(20)}$ (+0.139)	$E_{ACC}/M_{CTA(77)}$ (+114)	$MFC7_{(39)}$ (0.315)	$MFC7_{(38)}$ (0.328)	$MFC7_{(40)}$ (0.326)	$E_{AAC}/M_{CTA(64)}$ (0.117)	$E_{AAC}/M_{CTA(4)}$ (0.122)	$E_{AAC}/M_{CAT(282)}$ (0.138)
	$E_{AAC}/M_{CTA(64)}$ (+0.114)	$E_{AAC}/M_{CTA(13)}$ (+0.131)	$E_{ACC}/M_{CTA(125)}$ (+0.101)	$MFC8_{(56)}$ (0.225)	$MFC7_{(37)}$ (0.262)	$MFC5_{(24)}$ (0.304)	$E_{AAC}/M_{CTA(63)}$ (0.116)	$E_{AAC}/M_{CTA(20)}$ (0.134)	$E_{AAC}/M_{CAT(283)}$ (0.134)
	$E_{AAC}/M_{CAG(252)}$ (+0.121)	$E_{AAC}/M_{CTA(47)}$ (+0.130)	$E_{AAC}/M_{CAT(181)}$ (+0.111)	$MFC5_{(28)}$ (0.236)	$MFC7_{(41)}$ (0.254)	$MFC2_{(8)}$ (0.202)	$E_{ACC}/M_{CTA(106)}$ (0.116)	$E_{AAC}/M_{CTA(13)}$ (0.127)	$E_{AAC}/M_{CAT(265)}$ (0.118)
	$E_{AAC}/M_{CAG(253)}$ (+0.121)	$E_{AAC}/M_{CTA(53)}$ (+0.131)	$E_{AAC}/M_{CAG(261)}$ (+0.118)	$MFC2_{(3)}$ (0.251)	$MFC8_{(44)}$ (0.254)	$MFC8_{(52)}$ (0.179)	$E_{ACC}/M_{CTA(114)}$ (0.116)	$E_{AAC}/M_{CTA(53)}$ (0.126)	$E_{AAC}/M_{CAT(266)}$ (0.117)
	$E_{ACC}/M_{CTA(113)}$ (+0.110)	$E_{AAC}/M_{CTA(8)}$ (+0.121)	$E_{AAC}/M_{CAG(265)}$ (+0.134)	$MFC7_{(43)}$ (−0.298)	$MFC2_{(4)}$ (−0.174)	$MFC8_{(49)}$ (−0.290)	$E_{AAC}/M_{CAT(195)}$ (−0.100)	$E_{AAC}/M_{CAG(245)}$ (−0.128)	$E_{ACA}/M_{CAG(222)}$ (−0.111)
	$E_{ACC}/M_{CTA(114)}$ (+0.110)	$E_{AAC}/M_{CTA(9)}$ (+0.121)	$E_{AAC}/M_{CAG(262)}$ (+0.138)	$MFC3_{(15)}$ (−0.197)	$MFC3_{(10)}$ (−0.159)	$MFC6_{(36)}$ (−0.294)	$E_{AAC}/M_{CTA(41)}$ (−0.100)	$MFC7_{(381)}$ (−0.115)	$E_{ACA}/M_{CAG(224)}$ (−0.096)
	$E_{AAC}/M_{CTA(41)}$ (−0.113)	$E_{AAC}/M_{CAT(212)}$ (−0.111)	$E_{AAC}/M_{CAT(221)}$ (−0.103)	$MFC5_{(26)}$ (−0.194)	$MFC5_{(28)}$ (−0.165)	$MFC7_{(41)}$ (−0.218)	$E_{AAC}/M_{CAT(197)}$ (−0.090)	$E_{AAC}/M_{CAT(212)}$ (−0.110)	$E_{ACA}/M_{CAG(223)}$ (−0.094)
	$E_{ACA}/M_{CAG(127)}$ (−0.105)	$E_{AAC}/M_{CAT(213)}$ (−0.106)	$E_{AAC}/M_{CAT(222)}$ (−0.114)	$MFC8_{(49)}$ (−0.192)	$MFC6_{(35)}$ (−0.164)	$MFC8_{(50)}$ (−0.220)	$E_{AGC}/M_{CAA(320)}$ (−0.091)	$E_{AAC}/M_{CAT(213)}$ (−0.110)	$E_{ACA}/M_{CAG(221)}$ (−0.093)
	$E_{AAC}/M_{CAT(195)}$ (−0.112)	$E_{AAC}/M_{CAG(245)}$ (−0.128)	$E_{AAC}/M_{CAT(224)}$ (−0.104)						
	$E_{AAC}/M_{CAT(197)}$ (−0.101)								

(0.94) for SSR marker systems (Table 3). According to the formula used, the high value of MI calculated for the AFLP assay derives from its high effective multiplex ratio. This characteristic makes the AFLP marker system suitable for estimating genetic diversity level in breeding populations. The QND is the most important measure for the genebank curators and managers as they like to have genotyping data for their material that can be documented and handled easily in their database as well as can be communicated in terms of 'molecular passport data' among different genebanks across the globe (Varshney et al. 2007). In the whole, we recommend the utilisation of SSR markers for genotyping the genebank materials as suggested by the QND estimated for the SSR markers (0.75) and AFLP (0.093). In contrast, the QND for AFLP markers is the lowest as it is very difficult to interpret and document the AFLP genotyping data in genebank databases as suggested by Varshney et al. (2007).

Genetic Distances and Relationships Among Cultivars

AFLP Polymorphisms

To estimate the genetic relatedness among accessions, pairwise distance matrix was obtained based on the formula of Nei and Li's (1979). Similarity values were in good agreement with previously available data concerning the pedigree of accessions. Based on the 342 AFLP markers, estimates of the genetic distance exhibited values ranged from 0.04 to 0.59 with a mean of 0.32 signifying that the genotypes studied are characterised by large divergence at the DNA level. The smallest genetic distance value of 0.04 was scored between 'Soltani1' [SL1] and 'Kahli1' [KH1]. However, 'Wahchi' [WH] and 'Baghli' [BGL] were most divergent, with the maximum genetic distance of 0.59. All others have displayed different middle levels of genetic similarity.

The AFLP UPGMA dendrogram portioned the 38 tested cultivars into two major clusters (Fig. 2a). The first cluster (I) contained 'Makhbech' [MK], 'Hammouri' [HR], 'Zaghoubi' [ZH] and 'Baghli' [BGL] cultivars. All the remaining accessions are ranged in the second cluster (II) that exhibited two secondary ramifications. The first labelled (II-1) monophyletic branch consisted of 'Khadhouri' [KD] cultivar, while the second ramification (II-2) is composed of the remaining accessions.

The PCA of the AFLP data (Fig. 3) produced a picture similar to the one given by the cluster analysis. Up to 26.55% of the total variation was explained by the first three axes, which accounted for 9.99, 9.06 and 7.48, respectively, of the variation observed (Table 4). Figure 3 and Table 4 show AFLP markers that contribute mostly to

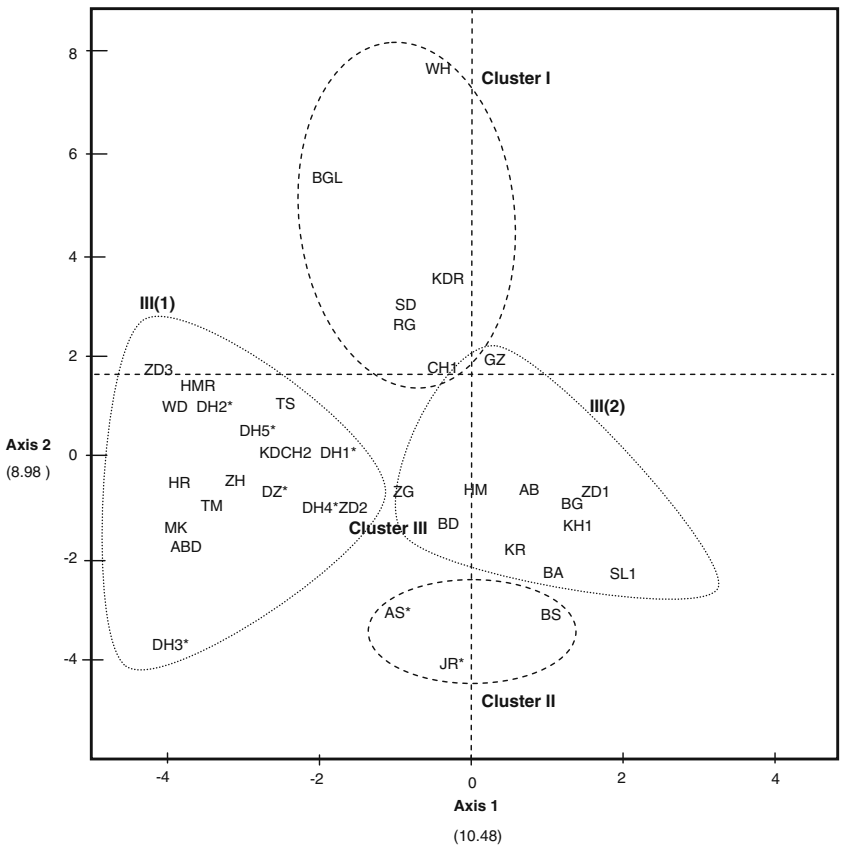

Fig. 4 Plot of 38 Tunisian fig genotypes according to the two first axes of the principal component analysis (PCA; 19.46% of the global diversity) based on 57 simple sequence repeats (Table 1 for cultivar codes; *asterisks*, male trees)

the observed clustering are markers positively defined axis 2 for cluster I and markers correlated negatively to axis 1 for sub cluster II-1 and sub cluster II-2.

SSR Polymorphism

A genetic distance matrix among accessions ranged from 0.017 to 0.546 with a mean of 0.281. The smallest distance value was observed between 'Zidi 1' [ZD1] and 'Baghali' [BG] accessions, which appear as the most similar accessions and closely regrouped. The maximum distance value of 0.546 was obtained between 'Dhokkar 3' [DH3] and 'Wahchi' [WH] cultivars. The cluster analysis using UPGMA based on genetic distances from SSR marker analysis revealed that the 38 genotypes can be divided into three main groups (Fig. 2b). The first group labelled (I) include 'Sawoudi' [SD], 'Chetoui1' [CH1], 'Baghli' [BGL], 'Rogaby' [RG], 'Wahchi' [WH] and 'Khadhri' [KDR] cultivars. The second group contains the two caprifigs of Sahel 'Jrani' [JR] and 'Assafri' [AS], as well as the cultivar 'Besbessi' [BS]. The third group exhibited two sub-clusters labelled III (1) and III (2) include all remaining accessions. In order to ascertain the accuracy of this assumption, data were computed to perform a PCA analysis. Results are summarised in Table 4. The three first axes accounted for 27.64% of the total variability. Figure 4 illustrates the distribution of cultivars according to the first two components (axis 1 and axis 2). The three major clusters and the two sub-clusters from the group analysis appeared to be well detached. Note that SSR markers correlated to axis 1 contribute the most to differentiate between clusters I and II. On the other hand, markers defined axis 2 contributes to define the two sub-clusters III1 and III2.

In conclusion, using SSR or AFLP data, caprifigs ecotypes namely [DH1], [DH2], [DH3], [DH4], [DH5], [JR], [AS] and [DHZ] are mixed with common fig accessions. Thus, we may assume that no correlation is observed in relation to the sex of trees. In addition, we assume that a typical continuous genetic diversity characterises the local fig germ plasm. This assumption is supported strongly by the fact that the cultivars studied are clustered independently from their geographical origin. Taking into account both *uniferous* and *biferous*, fig genotypes aggregated together in the same cluster indicating a possible common origin. This is in agreement with the monoecious origin of *Ficus* that has evolved into two gynodioecious forms as suggested by Machado et al. (2001). It is important to note that similar data have been reported in Tunisian figs using other molecular markers such as RAPDs, ISSRs, RAMPOs, ribosomal (ITS) and chloroplast data (Chatti et al. 2004b, 2007; Salhi Hannachi et al. 2006; Baraket et al. 2009a, b).

Polymorphisms of Fig Cultivars Based on Pooled SSR and AFLP Markers

The AFLP and SSR data were combined into a single analysis for a total data matrix of 399 polymorphic bands. These combined data produced genetic distances ranging from 0.051 to 0.56 with a mean of 0.30. 'Soltani 1' [SL1] (Sahel) and 'Kahli 1' [KH1] (Sahel) are the most closely related accessions (0.051), whereas 'Makhbech' [MK] and 'Wahchi' [WH] are the most distantly related ones (0.56). The clustering obtained is indistinguishable for clustering observed using AFLP data particularly the distinction of the two main groups that comprise either domestics or caprifigs (Fig. 5). In fact, the distribution of the variability showed by the bi-plot 1–2 of the PCA (24.47%) suggests that the genetic diversity was typical continuous since there is no distinctiveness of accessions clusters (Fig. 6), signifying their dispersion is made independently either from the geographical origin, the horticultural classifications or the sex of trees.

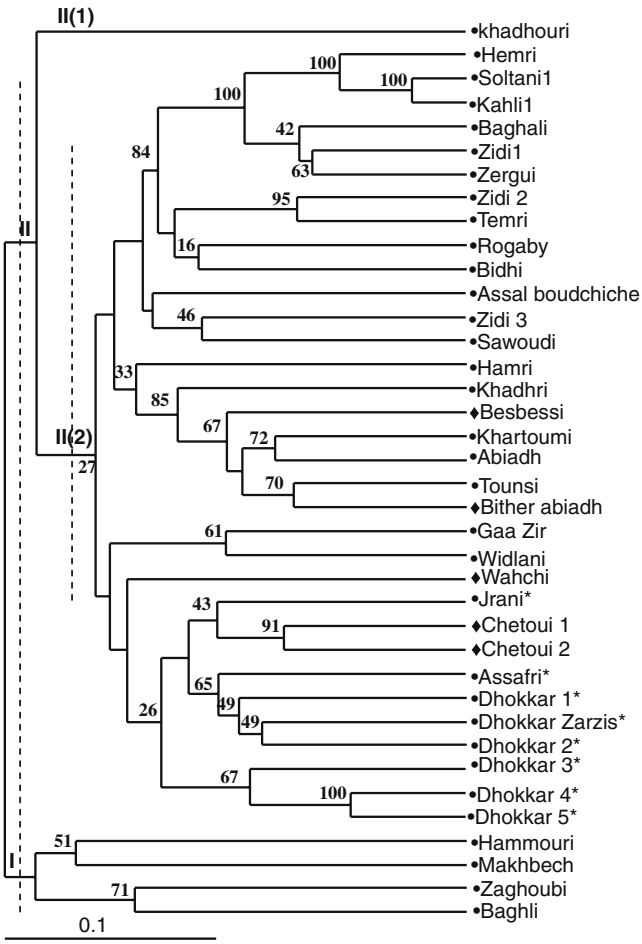

Fig. 5 Dendrogram of 38 fig genotypes by simple sequence repeat (SSR) and amplified fragment length polymorphism combined data analysis. *Asterisks*, male trees; *circles*, Unifere cultivars; *diamonds*, Bifere cultivars

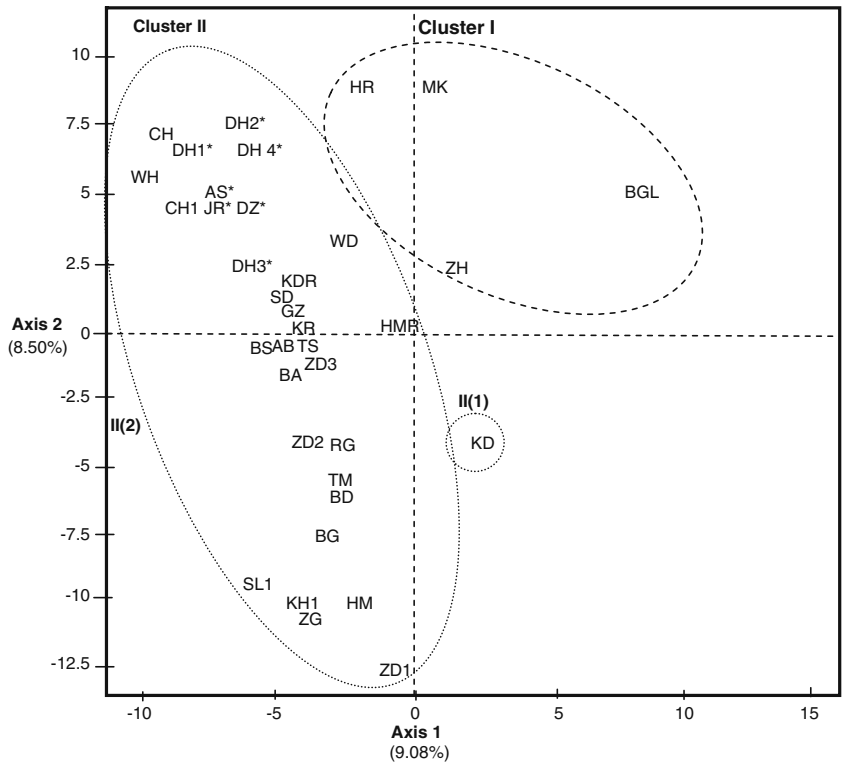

Fig. 6 Plot of principal component analysis (17.58% of the total diversity) of 38 Tunisian fig accessions based on simple sequence repeat and amplified fragment length polymorphism pooled markers (Table 1 for cultivar codes; *asterisks*, male trees)

Partitioning of Genetic Variation

AMOVA analysis allowed the partitioning of the genetic variation among and within groups using AFLP, SSR and combined data (AFLP&SSR). We estimated the variance components to assess which marker contributes the most to the genetic diversity within-group or among-group level (Table 5). The among-group variance components were very low which indicate that the genetic background attributable to the geographical origin contributes slightly to the observed genetic diversity. In addition, AMOVA analysis indicates that more than 95.17% of total genetic diversity by AFLP, SSR and aggregated markers (AFLP&SSR) are distributed within groups, although only 3.13, 16.41 and 4.83 of the diversity are attributed to differences among regions. This low variability between regions was also reported in European and Asian fig varieties using ISSR, RAPD and SSR markers as reported

Table 5 Results of analysis of molecular variance conducted on amplified fragment length polymorphism, simple sequence repeat and combined data (AFLP&SSR)

Source of variation	*df*	Sum of squares	Mean squares	Variance components	Percentage of variation	*P* value[a]
AFLP	4	244.743	61.186	1.607		<0.001
Among group					3.13%	
Within group	33	1,642.283	49.766	50.372	96.87%	<0.001
Total	37	1,887.026			Φst=0.031	
SSR	4	61.059	15.265	1.251		<0.001
Among group					16.41%	
Within group	33	210.335	6.374	6.373	83.59%	<0.001
Total	37	271.394			Φst=0.164	
AFLP and SSR	4	305.538	76.385	2.851		<0.001
Among group					4.83%	
Within group	33	1,852.119	56.125	56.124	95.17%	<0.001
Total	37	2,157.657			Φst=0.048	

df degree of freedom

[a] *P* value, significant test after 1,000 random permutations

by Ikegami et al. (2008) and in Tunisian collections using ISSR, RAPD, SSR and AFLP (Salhi Hannachi et al. 2005; Saddoud et al. 2007; Baraket et al. 2009b). On the other hand, SSR represents 16.41% among group variation, showing that SSR reflects considerably the variation depending on geography than AFLP and combined data. According to Salhi Hannachi et al. (2005) and Ikegami et al. (2008), the low divergence between collections/groups can be explained using the occurrence of gene flow or common origin of the populations from which cultivars originated and the reproduction mode. In fact, life form and breeding systems can have significant influences on the genetic variation and its portioning (Hamrick and Godt 1996; Salhi Hannachi et al. 2005).

Correlation Between SSRs and AFLPs Matrices

To understand the degree of correspondence, if any, pairwise comparison of similarity matrices was carried out based on Mantel correspondence test. As reported in Fig. 7, the Spearmen's coefficient exhibited significant and higher positive correlations between AFLP data matrix and the AFLP&SSR matrix (s=0.9842 at P<0.05). The correlation between SSR and AFLP&SSR matrices was moderate (R= 0.2700 at P<0.05). However, lower and significant correlation was scored between AFLP and SSR markers (R= 0.1220 at P<0.05). In particular, genetic data of the used markers were found to be slowly correlated as demonstrated by the Spearman correlation coefficient R=0.1221 (P<0.05) for AFLP and SSR. A same result was founded as suggested by Woodhed et al. (2005) in *Althyrium distentifolium* species. The low number of SSR markers compared to the high number of AFLP bands can explain this result. On the other hand, Spearman test shows also low value of 0.2745 (P<0.05) for SSR and combined AFLP&SSR dataset despite the small number of SSR markers compared to the AFLP ones. This finding demonstrates the effectiveness of SSR and AFLP considered separately and pooled markers in genetic diversity study and characterization of Tunisian figs. The significant correlations between genetic distances matrices show that AFLPs were better linked to the combined dataset than were SSR markers. A high degree of congruence was

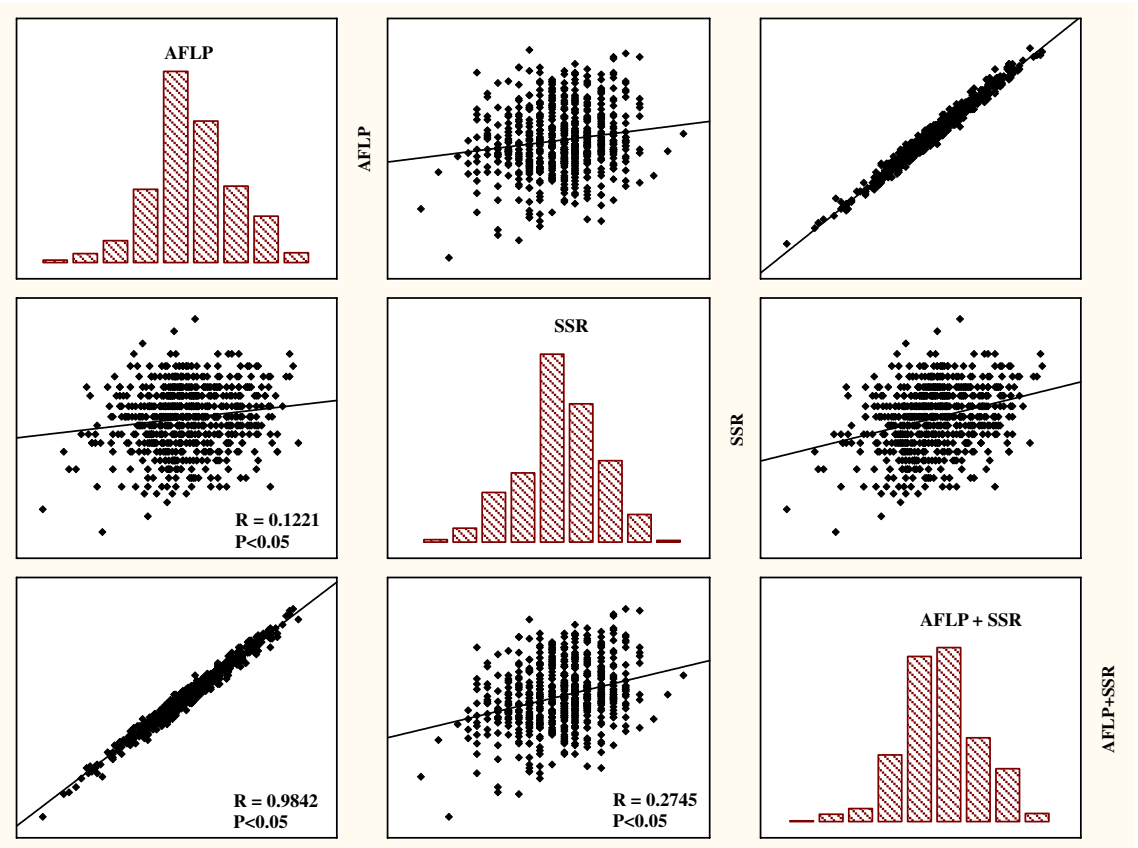

Fig. 7 Spearman's correlation coefficient (R) estimated between genetic distances matrix for 38 fig cultivars as calculated using data from amplified fragment length polymorphism (AFLP), simple sequence repeat (SSR) and combined data (AFLP&SSR) and probability test calculated at the 0.05 level (*asterisks*, significant). Two-dimensional histograms present a graphical representation of the frequency distribution of genetic distances generated using the selected marker. Two-dimensional scatterplots, which are to be the graphical equivalent of the correlation matrix, are shown for each pair of markers; the *linear regression line* is also shown in each scatterplot

obtained among compared cultivars when using AFLP and pooled AFLP&SSR data. Significant correlations in the test used underline this conclusion (R=0.9842, P<0.05) for a combined AFLP&SSR and AFLP dataset. The low number of SSR markers may be one of the reasons for observing a high correlation between AFLP and combined AFLP&SSR. Several studies have examined correlation between genetic distances matrices obtained by different kinds of molecular markers applied in various species. Powell et al. (1996) reported similar genetic relationships in wild and cultivated soybean using four marker systems. Russel et al. (1997) found that restriction fragment length polymorphism and AFLP were correlated in barley. Pejic et al. (1998) reported similar results in maize inbred lines. RAPD and AFLP revealed similar relationships among clones of various willow species (Barker et al. 1999), *Vigna angularis* accessions (Yee et al. 1999), pepper inbreds (Lefebvre et al. 2001), rice genotypes (Virk et al. 2000) and apple cultivars (Goulao et al. 2001). The AFLP technology showed a much higher multiplex ratio than the RAPD technique (42.4 vs. 6.1). The eight combinations of EcoRI/MseI primers were enough to clearly distinguish between all fig clones (Cabrita et al. 2001), and results from both AFLP and SSR datasets were very similar and suggested that *Erianthus rockii* was distinct from *Erianthus* and *Saccharum* species as mentioned by Cai et al. (2005).

Conclusions

In conclusion, our results demonstrate that the two types of marker assays have different characteristics, and the most important criteria determining choice of an assay are informativeness and easiness of genotyping (Powell et al. 1996). On the other hand, this study demonstrates that the Tunisian fig genetic diversity is important as demonstrated by the use of AFLP and SSR markers. The molecular markers, especially both dominance and co-dominance markers utilisation, can be crucial for identification and estimation of the relatedness at the variety level because they engender different classification results based on their respective characters. In fact, AFLP markers had a higher effective multiplex ratio value (56.9), and the estimated MI parameters revealed their much higher relative information content in comparison to SSR. Additionally, the result suggests that the AFLP markers are the best choice for the evaluation of diversity level and assessing the genetic relationships among fig cultivars with high accuracy. The AFLP system presents good levels of precision in its genetic estimates and single crosses prediction. AFLP also highly corroborates with results obtained using the SSR system and is a fast and reliable system capable of supporting a multiplex approach not requiring previous knowledge of DNA sequencing. Nevertheless, the two marker systems suggested comparable descriptions of diversity since the two kinds of markers show that the genetic diversity is structured independently either from the geographical origin, horticultural type and sexes of trees. In addition, low and significant correlation was detected among genetic matrices which allowed the efficacy and the utility of the two marker systems. An understanding of both the genetic diversity and the population structure of *F. carica* in Tunisia can also provide insight into the conservation and management of this species.

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