Trees
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ORIGINAL ARTICLE

Conserved DNA-derived polymorphism as a useful molecular marker to explore genetic diversity and relationships of wild and cultivated Tunisian figs (*Ficus carica* L.)

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Abstract

Key message **The first insight into the genome of *Ficus carica* L. with a gene target marker (conserved DNA-derived polymorphism (CDDP)) and assessment of genetic diversity mostly related to functional domains of plant genes.**

Abstract To improve the molecular database of *Ficus carica* L. species, we report for the first time the use of conserved DNA-derived polymorphism (CDDP) as a gene-targeted marker to assess molecular diversity, and establish relationships among 62 Tunisian cultivated and wild fig trees. The mapping process for the in silico analysis of CDDP primers against the whole *F. carica* genome cv Dottato verified the specificity of the CDDPs and the stringency of PCR conditions. Overall, a set of twelve CDDP primers were tested revealing 200 markers. Based on the polymorphic information content (PIC = 0.90), resolving power (*R*p = 8.13) and the level of polymorphisms (98.04%) CDDP markers were found to highly discriminant and informative compared to other non-targeted methods. The UPGMA dendrogram revealed that Tunisian figs could be differentiated into three main groups, which was also supported by the principal coordinate analysis. The analysis of molecular variance (AMOVA) suggested that the maximum genetic variation was within groups (86.10%) with less variation among groups (19%) indicating that there is a limited diversity that distinguishes fig groups. Here, we present the first report in which a targeted DNA region molecular marker successfully clustered the Tunisian fig germplasm depending on the sex, the botanical classification of figs and consistently in agreement, with their geographic origin). The results highlight that the CDDP markers are able to characterize wild and cultivated *Ficus carica* L. species and provide a new valuable tool for further genome investigation and will guide the development of conservation and management strategies for existing fig tree germplasm.

Keywords *Ficus carica* L. · CDDP markers · Genetic diversity · Wild · Cultivated · Tunisia

Introduction

The fig tree (*Ficus carica* L.) belongs to *Ficus* genus of the Moraceae family (Flaishman et al. 2008). It is a gynodioecious species with two sexual forms: male trees (Caprifig) and female trees, showing a diploid genome configuration with 26 chromosomes (Essid et al 2015; Knap et al. 2016). Since only subspecies *caprificus* (Male fig) produce pollen for commercial fig plantings (Marcotuli et al. 2020), and their figs host the pollinator, *Blastophaga psenes* L. (Achtak et al. 2010). The subspecies *domestica* (domesticated, common, or female fig) is functionally dioecious and is the only producer of edible figs (Falistocco 2016; Aljane and Ferchichi 2009).

The edible fig is the first crop grown in the world (Kislev et al. 2006). The East Mediterranean region (Turkey, Syria and Saudi Arabia) was considered as the origin of the *Ficus carica* L. species from which its cultivation expanded to the whole Mediterranean region (Kislev et al. 2006). According to the Food and Agriculture Organization of the United Nations, nowadays fig trees are grown in 55 countries around

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the world and yielded about 1.1 million tons of fresh figs while Tunisia is the ninth fig fruit producer in the worldwide (FAOSTAT 2019).

In Tunisia, local fig cultivars are numerous and well adapted to the agro ecological conditions, thus representing an important case for study genes linked to biotic and abiotic stress (Aljane and Ferchichi 2010). Moreover, wild fig trees are abundant, which propagate exclusively by seeds, (Falistocco 2016) and mostly present between rocks along the riverbanks and in steep-sloped valleys where seeds are easily disseminated by birds (Ben Abdelkrim et al. 2015; Falistocco 2020). However, the local fig resources are currently highly threatened by genetic erosion due to various stresses including intensive urbanization, monovarietal cultures, attacks of pathogens (such FMD: Fig Mosaic Disease), and lack of selected caprifigs (Mars 2003; Salhi Hannachi et al. 2004; Caliskan and Polat 2012). Hence, there is an urgent need to maintain the present fig resources as much as possible, not only for the long-lasting survival of the species, but also to guarantee enough variability for breeding programs (Esquinas Alcazar 2005; Caliskan and Polat 2012). To date, the genetic diversity level of cultivated figs has widely evaluated, but wild figs and caprifigs still remain to investigate. (Ben Abdelkrim et al. 2015; Knap et al. 2016; Essid et al. 2015; Caliskan et al. 2018).

Several studies have been conducted on *Ficus carica* gene expression (Freiman et al. 2015; Chai et al. 2017; Marcotuli et al. 2020), functional genomics (Ikegami et al. 2013; Mori et al. 2017; Zambrano et al.2017), and transcriptome sequencing (Freiman et al. 2014; Zambrano et al.2017; Cui et al. 2019; Vangelisti et al. 2019; Wang et al. 2019). Genes encoding for FcMADS-box transcription factors and ethylene-response factors (ERFs) such as ERF1 were found to be closely associated and strongly up regulated during fig maturation and fruit ripening, respectively (Ikegami et al. 2013; Freiman et al. 2014, 2015). In addition, Ikegami et al. (2013) showed inter-type variations in B- and C-class MADS-box gene homologs, which are believed to be involved in sexuality. Newly, Vangelisti et al. (2019) demonstrated the over-expression of ABA-dependent genes (e.g., MYB and WRKY) known to be involved in salt stress response in *F. carica*.

Nowadays, thanks to available genomics data resources, several molecular markers encoding candidate genes have been developed and more studies were associated to these gene-targeted markers such as Conserved DNA derived polymorphism (CDDP) (Collard and Mackill 2009). The CDDP technique is based on a single primer amplification reaction; primers are designed precisely to target conserved sequences of plant genes. In fact, 15- to 19 oligonucleotides primers for PCR as forward and reverse primers were used under a high annealing temperature (50 °C) to amplify reproducible and polymorphic markers when the DNA sequence of the conserved region was reverse complemented (Collard and Mackill 2009). PCR amplification using gene-specific primers can targets conserved sequences of gene families present in multiple copies in the plant genome so given the number of conserved gene regions, many regions could be targeted by CDDP technique. Therefore, CDDP markers can easily generate functional markers linked to the related plant phenotype (Poczai et al. 2013). Since highly conserved DNA regions share the same priming site that is extended in the genome across different plant species but differs in their genomic distribution, variation can be detected as length polymorphism within these regions (Poczai et al. 2013). Due to their dominant nature, polymorphism and high reproducibility, CDDP markers have been used to resolve homonymy and synonymy problems, explore diversity and describe relationships among cultivars of several species such as: *Chrysanthemum* (Li et al. 2013), *Paeonia* (Chen et al. 2018) *Triticum* (Seyedimoradi et al. 2016), *Phoenix dactylifera* (Atia et al. 2017), *Rosa rugosa* (Jiang and Zang 2018), *Anthurium andraeanum* (Saidi et al. 2018), *Carthamus tinctorius* (Talebi et al. 2018), *Pistacia vera* (Aouadi et al. 2019), *Elaeagnus macrophylla* (Wang et al. 2020), *Salix taishanensis* (Liu et al. 2020) and recently in *Musa* L. (Okeh Igwe et al. 2021).

In this work genomic regions have been amplified by short primers designed on genes like WRKY, MYB, ERF, KNOX, MADS, and ABP1, which are mostly involved in regulation of plant development processes or biotic and abiotic stresses response. The study herein reports for the first time the use of CDDP technique to develop molecular tools as a gene-targeted marker to assess genetic diversity and establish relationships among 62 Tunisian cultivated and wild figs trees. The provided data can be used to preserve fig germplasm and develop an efficient breeding program, propose recommendations for the development of conservation strategies for these resources in the face of the climate change predicted for the upcoming years.

Materials and methods

Plant materials

Sixty-two Tunisian fig accessions, including 11 male figs or caprifigs (five wild pollinizers and six cultivated) and 51 female figs (24 wild and 27 cultivars) were collected from different geographical locations through Tunisia (North, Center, South and Island of Kerkennah) (Table 1, Fig. 1). For the cultivars, the sampling strategy was conducted by questioning farmers and for wild fig trees, our sampling was carried out according to some ecological criteria such as the soil type, humidity and temperature of prospected sites. These criteria specify the natural habitats fig distribution,

Table 1 Listing of *Ficus carica* L. accessions included in this study indicating their geographic origin, sex (common or caprifig), type (wild or cultivars)

Code	Cultivar name/label individual's	Sex type	Locality	Region
Caprifig groups (cultivated and spontaneous)				
1	Dhokkar	♂	Rafraf	North East
2	Caprifig F207	♂	Neftasouani	South West
3	Caprifig F208	♂	Neftasouani	
4	Caprifig F209	♂	Neftasouani	
5	Wild pollinator P4 Zag tir	♂	El Alia	North East
6	Wild pollinator P5	♂	Ras jebal	
7	Wild pollinator P10	♂	Bejjou	
8	Wild pollinator P11	♂	Bejjou	
9	Wild pollinator P12	♂	Bejjou	
10	Jrani	♂	Chott Mariem Collection (I.S.A)	East(Sahel)
11	Assafri	♂		
Cultivated fig group				
12	♀	♀	Rafraf	North East
13	♀	♀	KerkenahIsland	Center East
14	♀	♀	KerkenahIsland	
15	Kahla Cherki	♀	KerkenahIsland	
16	KhédhriHorr	♀	Kesra	North West
17	TirriHorr	♀	Kesra	
18	SafriGuaress	♀	Kesra	
19	Dam Froukh	♀	Kesra	
20	Soltani	♀	Germplasm collection of the High Agronomic Institute (I.S.A) of Chott-Mariem	East (Sahel)
21	BighBeghal	♀		
22	Zidi	♀		
23	Besbessi	♀		
24	Goutti	♀		
25	Bidhi	♀		
26	Bither AB	♀		
27	Hemri	♀		
28	taganimt	♀		
29	HobbiAbyath	♀	'Centre Régional de Recherches en Agriculture Oasienne (CRRAO)' Degache	SouthWest
30	Boumarra	♀		
31	Zidi jamrou	♀		
32	Bsisassal	♀		
33	KhlitJbéni	♀		
34	BasoulElkhadem	♀		
35	Hammi	♀		
36	AounkEhmém	♀		
37	khzéfi	♀		
38	Limi	♀		
Wild fig group				
39	FAS 11	♀	El Mida	North West (CapBon)
40	FAS 12	♀	El Mida	
41	FAS 13	♀	El Mida	
42	FAS 14	♀	El Mida	
43	FAS 22	♀	Haouaria	
44	FAS 23	♀	Haouaria	
45	FAS 25	♀	Haouaria	
46	FAS 27	♀	Haouaria	

Table 1 (continued)

Code	Cultivar name/label individual's	Sex type	Locality	Region
47	FAS 37GE	♀	Ghar el milh	North East
48	FAS 45	♀	Ghar el milh	
49	FAS 46	♀	Ghar el milh	
50	FAS 47	♀	Rafraf	
51	FAS 49	♀	Rafraf	
52	FAS 55	♀	Rafraf	
53	FAS 28	♀	Kerkenah Island	Center East
54	FAS 30	♀	Kerkenah Island	
55	FAS 34TN	♀	Kerkenah Island	
56	FAS 35TN	♀	Kerkenah Island	
57	FAS 63	♀	Siliana	North West
58	FAS 65	♀	Siliana	
59	FAS 88	♀	Kesra	
60	FAS 90	♀	Kesra	
61	FAS 93	♀	Kesra	
62	FAS 96	♀	Kesra	

between rocks along the riverbanks, steep-sloped valleys, and islands as reported by Nabli (1991) and Aljane et al. (2008).

DNA extraction and PCR amplification

Total genomic DNA was isolated from young leaves sampled from adult trees following Dellaporta et al. (1983) method. The DNA concentrations were quantified using a Qubit^{R} fluorometer (purchased from Invitrogen) and their quality was checked on a 0.8% agarose gel electrophoresis (Sambrook et al. 1989). DNAs were diluted to a final concentration of 30 ng $\mu L^{-1}$with ultrapure water and stored at − 20 °C until used.

Twelve CDDP primers corresponding to conserved regions of six genes: WRKY, MYB, KNOX, ERF, MADS and ABP1 were selected for PCR amplification following Collard and Mackill (2009) (Table 2). CDDP-PCR mixture contains approximately 30 ng/µL of template DNA, (10X) PCR buffer, 25mMMgCL_2, 20 mM deoxyribonucleotide triphosphate (dNTP), 0.5 U of Taq polymerase and 10 mM of each primer. The reaction volume was adjusted to 25 µL withMilli-Q water (Millipore, Europe). The PCR was performed in a thermal gradient cycler TProfessional TRIO Thermocycler (Biometra, Germany)begins with an initial denaturation step at 94 °C for 3 min, followed by 35 cycles (DNA denaturation at 94 °C for 1 min, optimal annealing temperature for 1 min, elongation at 72 °C for 2 min) with a final extension at 72 °C for 5 min (Collard and Mackill 2009). All The amplified products were resolved on 1.5% agarose electrophoresis in 0.5X TBE buffer (8 mM Tris-borate, 8.9 mM boric acid, 8 mM EDTA), stained with ethidium bromide (0.5 mg ml^{-1}) and visualized under UV light (Bio-Rad, USA) and photographed using a Gel Documentation System (Bio-Rad Gel Doc 2000, USA).

In silico estimation of expected number of bands

To verify the specificity of CDDPs used in the PCRs, the *F. carica* genome cv Dottato (GCA_009761775.1, Usai et al. 2020) available on the NCBI site (https://www.ncbi.nlm.nih.gov/) was downloaded and the oligonucleotides were searched against the whole genome using the "Find binding sites and create fragments" function of CLC Genomics Workbench v21 (CLC-BIO, Aarhus, Denmark). Several analyses were carried out to find the specific values for the "minimum number of base pairs required for a match" and "number of consecutive base pairs required in 3'-end" parameters. In particular, for 15 bp_long CDDPs these parameters were set to 10 and 7 bp, 11 and 8 bp, 12 and 9 bp, respectively. Concerning, CDDPs with length of 17 and 18 bp, the same parameters were set to 11 and 8 bp, 12 and 9 bp, 13 and 10 bp, respectively. The PCR fragment length range was set between 200 and 3500 bp.

Data scoring and diversity measures

For each CDDP primer, the amplified bands were scored according to the presence (1) or absence (0) of the same size, the data were then transformed into a 1/0 binary matrix. Here we considered only clear and reproducible bands

Fig. 1 Map of Tunisia and geographical distribution of *Ficus carica* L. samples studied

amplified in data scoring. Description about score evaluation is described as follows.

The total number of amplified bands (TNB) and the number of polymorphic bands (NPB) were calculated to value the efficiency of each CDDP primer and detect polymorphic loci among the accessions. The ability of the most informative primers to differentiate between fig accessions was evaluated by calculating their resolving power (*R*p) based on the formula of Prevost and Wilkinson (1999):

$$Rp = \sum Ib,$$

where Ib is the band informativeness with $Ib = 1 - (2 \times |0.5 - p|)$ and where p is the proportion of genotypes containing the band I.

The resolving power is based on the distribution of detected bands within the sampled genotypes.

The polymorphism information content (PIC), which measures the ability of a marker to detect polymorphisms, was estimated according to Smith et al. (1997) using the formula:

$$PIC = 1 - \sum Pi2,$$

where Pi is the frequency of the ith allele for all genotype obtained.

The similarity matrix UPGMA dendrogram and PCoA analysis

To establish the relationships among Tunisian fig accessions, a similarity matrix using the Ochiai coefficient (Ochiai 1957) was calculated. According to the similarity matrix data, a dendrogram based on cluster analysis using the Unweighted Pair Group Method with Arithmetic Means (UPGMA) algorithm and Principal Coordinate Analysis (PCoA) were performed with the Paleontological Statistics (PAST) software Version 3.25 (Hammer et al. 2001).

Hierarchical analysis of molecular variance (AMOVA)

AMOVA was performed with GenAlEx 6.51 program (Peakall and Smouse 2012) based on the CDDP markers. The AMOVA components were used as an estimation of molecular diversity at two hierarchical levels to examine the differences among and within Tunisian *Ficus carica* groups. The significance of P values ($P < 0.001$) was tested non-parametrically, after 1000 random permutations.

Results

In silico results

The specific parameters for the in silico verification of CDDPs against the whole *F. carica* genome cv Dottato were: (a) minimum ten base pairs required for a match and minimum seven consecutive base pairs in 3′-end for CDDPs with length of 15 bp, and (b) minimum 11 base pairs required for a match and minimum 8 consecutive base pairs in 3′-end for CDDPs with length of 17 and 18 bp. Stricter parameters (i.e. greater number of base pairs required for a match) led to the identification of a much smaller number of genomic targets than the number of observed bands, with a mean value of 2.42 and 0.84 putative virtual fragments. The mapping process based on the chosen parameters resulted in a mean value of 46.33 putative virtual fragments (Supplementary Table S1). The result is in line with those achieved in

Table 2 Characteristics of conserved DNA-derived polymorphism (CDDP) primers used to assess genetic diversity and summary of polymorphism generated in *Ficus carica* L.

Gene	Gene function	Primer name	Primer sequence (5′–3′)	Length	% GC	T_a	Size range (bp)	TNB	NPB	PPB (%)	PIC	*R*p
WRKY	Transcription factor for development and physiological roles	WRKY-F1	TGGCGSAAGTACGGCCAG	18	67	50	3000–200	18	18	100	0.913	8.387
		WRKY-R3	GCASGTGTGCTCGCC	15	73	49	2500–300	18	17	94.44	0.912	8.065
		WRKY-R2B	TGSTGSATGCTCCCG	15	67	50	3000–200	21	21	100	0.938	11.87
		WRKY-R3B	CCGCTCGTGTGSACG	15	73	49	2500–300	11	9	81.82	0.874	4.516
YB	Unknown (implicated in secondary metabolism, abiotic and biotic stresses, cellular morphogenesis)	Myb1	GGCAAGGGCTGCCGC	15	80	50	2500–300	24	24	100	0.948	14.483
ERF	Transcription factor involved in plant diseases resistance pathway	ERF1	CACTACCGCGGSCTSCG	17	77	50	2000–300	19	19	100	0.914	7.322
		ERF3	TGGCTSGGCACSTTCGA	17	65	50	2500–200	20	20	100	0.934	11.516
KNOX	Homeobox genes that function as transcription factors with a unique homeodomain	KNOX-2	CACTGGTGGGAGCTSCAC	18	67	50	2000–500	12	12	100	0.892	6.193
		KNOX-3	AAGCGSCACTGGAAGCC	17	65	48	3500–300	19	18	94.73	0.902	8.225
MADS	Involved in controlling floral organ initiation and development	MADS-4	CTSTGCGACCGSGAGGTG	18	72	50	2000–400	16	16	100	0.87	5.354
ABP1	Auxin-binding protein	ABP1-1	ACSCCSATCCACCGC	15	73	50	3500–300	17	17	100	0.906	7.677
		ABP1-2	ACSCCSATCCACCGG	15	73	49	3000–800	9	9	100	0.819	4.032
Total								204	200	-	-	97.64
Mean								17	16.66	98.04	0.90	8.13

GC (%) guanine–cytosine content (%), *Ta* the annealing temperature of CDDP primers, *TNB* total number of bands, *NPB* number of polymorphic bands, *PPB* percentage of polymorphic bands, *Rp* resolving power, *PIC* polymorphism information content

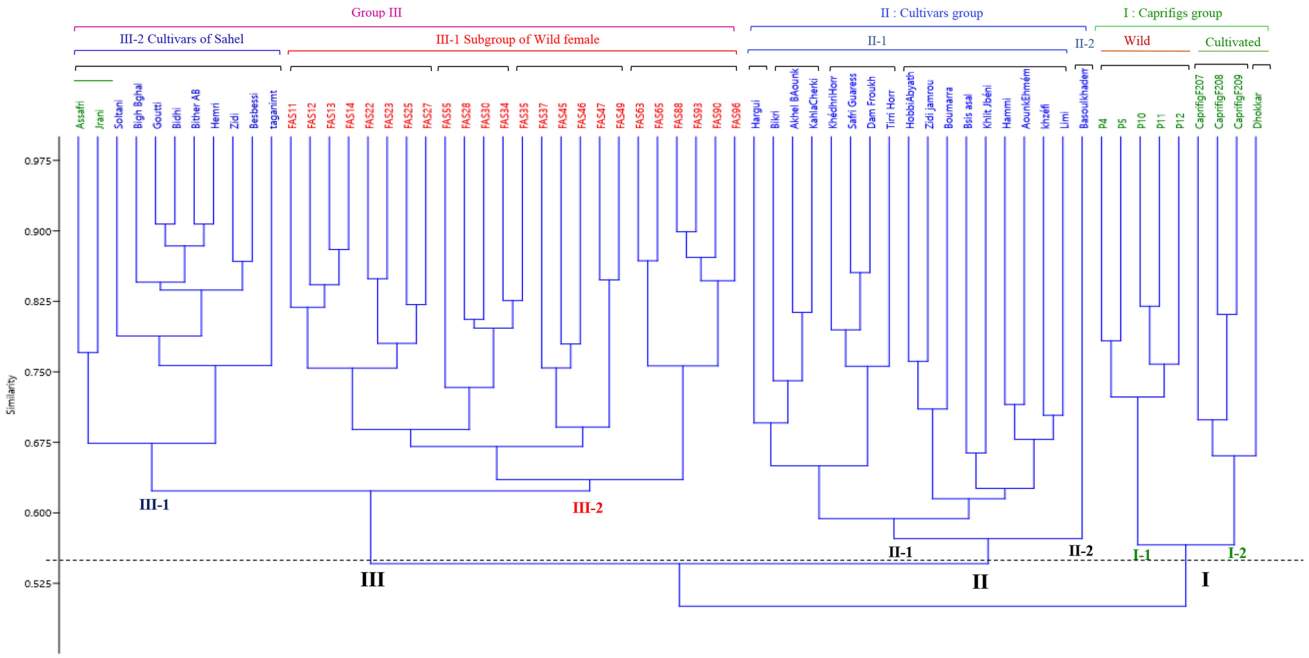

Fig. 2 UPGMA dendrogram obtained from cluster analysis of 62 Tunisian fig trees based on Ochiai's similarity index matrix showing the relationships between the three different fig groups. The colors correspond to the three fig groups studied (red: wild female; bleu: cultivated female and green: wild and cultivated caprifigs). The closing brackets colored in black represent the fig samples belonging to the same geographical distribution

PCR experiments, considering multiple factors that come into play during PCR reactions. They cannot be adequately taken into consideration into an in silico analysis (e.g. temperatures, GC contents, primer and salt concentration, etc.). It is also important to bear in mind that the reference genome belongs to a different cultivar from those analyzed. Hence, the number of putative fragments obtained from the in silico analysis should support the specificity of the CDDPs and the stringency of PCR conditions.

Diversity results

For the analysis of molecular polymorphism in *Ficus carica* L. species, 12 CDDP primers were designed on conserved regions of six genes involved in several features derived from the genomic DNA of plants. As illustrated in Table 2, these primers revealed 204 bands with an average of 17 bands per primer. The number of polymorphic bands (NPB) is 200 in total, and varied from nine bands for the ABP1-2 to 24 for Myb1 primer with an average of 16.66 bands per primer, indicating that Myb1 is most efficient in the detection of polymorphism, against the ABP1-2primer which appears to be less effective since it yields the lowest number of polymorphic bands. The percentage of polymorphic bands (PPB) varied from 81.82% for WRKY-R3B primer to 100% for nine primers: WRKY-F1, WRKY-R2B, Myb1, ERF1, ERF3, KNOX-2, MADS-4, ABP1-1 and ABP1-2 with a rate of polymorphism of 98.04%. The size of the fragments generated varied from 200 bp for the primers WRKY-F1 and ERF3 to 3500 bp for ABP1-1 and KNOX-3 primer. The polymorphism information content (PIC) varied from 0.819 for the ABP1-2 primer to 0.948 for Myb1 primer with a mean of 0.90 (Table 2), which shows that the CDDP primers used are very discriminating. The resolving power (*R*p) varied from 4.032 for the ABP1-2 primer to 14.483 for Myb1 with an average of 8.13 and the collective value of the resolving power is 97.64 (Table 2) indicating that these loci were very informative and with high reproducibility.

Cluster analysis

The UPGMA clustering dendrogram of the 62 fig accessions was constructed based on similarity coefficient of Ochiai (1957). The similarity indices ranged between 0.370and 0.970.The smallest was recorded between 'Kahla cherki' variety from Kerkennah Island and the wild pollinator 'P12' from Bizerte, which seem to be the most divergent. While 'Hemri' and 'Bither AB', 'Bidhi' and 'Goutti' cultivars maintained in Chott Mariem germplasm collection, displaying the highest distance, seem to be the most genetically similar accessions, and closely clustered, all the remaining fig accessions exhibited intermediate levels of similarity. The UPGMA dendrogram highlights the formation of three major groups (Fig. 2).

Cluster I gathered the male figs from northern and southern Tunisia except the two caprifigs ('Jrani' 'Assafri') housed in Chott Mariem collection from the Sahel region. This group is divided into two sub-groups, the first one (I-1) includes wild male pollinizers ('Zag tir P4', P5, P10, P11 and P12) collected from 'Bizerte' (Northeast of the country), and the second one (I-2) contains the cultivated caprifigs 'Dhokkar' from 'Bizerte' plus F207, F208 and F209 from 'Naftah' in the south.

Cluster II is homogeneous which assembles the cultivated female fig from the northeast, center and southwest. The cultivar 'Besoul Elkhadem' maintained in the 'Centre Régional de Recherches en Agriculture Oasienne (CRRAO) Degache' collection from southwest diverges significantly from the other cultivars of the same germplasm collection as well as all the other female cultivars and stands alone as a subgroup (II-1). However, the subgroup (II-2) contains the remaining cultivars from Degache collection clustered together with the remaining ones from the northeast, Kerkennah Island, and the center of the country.

Cluster III clustered all the wild fig accessions and the remaining cultivars together and formed a heterogeneous. This group is divided into two sub-clusters: a first heterogeneous sub-cluster (III-1) which grouped together all the accessions of Chott Mariem collection from Sahel and a second homogeneous sub-cluster noted (III-2) gathered all the wild fig populations. The sub-cluster III-2 is subdivided into two secondary ramifications. All the female cultivars housed in Chott Mariem collection formed the first one and the second ramification display the detachment of the two caprifigs 'Assafri' and 'Jrani' from all the female accessions (Fig. 2). Nevertheless, it seems that some small sub-clusters could be grouped consistently in agreement, in major part, with their geographic origin. For instance, the wild female cluster (III-2) comprises the wild population from the same locality: CapBon, Kerkennah Island, northeast and the center. The group of cultivars contains accessions from the center and the southwest of Tunisia. Finally, the group of caprifig ecotypes comprises the northeast group and the southwest fig ones. It is worthy of note that dendrogram clustering closely corresponded to the sex (common or caprifig) and the type (wild or cultivars) and consistently in agreement, in major part, with the geographic origin of the fig tree.

Principal coordinate analysis

The principal coordinate analysis (PCoA) based on CDDP data was also performed to get an alternative view of the relationships and distance between the 62 Tunisian *Ficus carica* L. genotypes. In fact, the first two principal axes accounted for 17.82% and 11.69% of the total variability (29.51%) (Fig. 3). The fig accessions were clearly classified into three groups, which was in agreement with the UPGMA clustering above. Indeed, the first group (I) is composed only of male figs. This proves their similarities and detachment from the other groups by the two axes 1 and 2 (except 'Jran' and 'Assafri'). The second group (II) contains the entire cultivated female fig, excluding all the cultivars of Chott Mariem collection that were closely related and overlapped with the wild fig group (III).

Hierarchical variance analysis results

The analysis of molecular variance (AMOVA) revealed significant differences among groups explaining 19% of the total variation and the remaining 81% within groups (Table 3). The moderate level of differentiation among the three groups is justified by the large common basis shared by fig types and exchange between them. This was recognized as a major factor to explain the partition of the observed diversity. These results demonstrate the presence of genetic diversity between the figs studied and underline the appearance of important gene flow between caprifig, wild, and cultivated Tunisian fig tree germplasm.

Discussion

Conserved DNA regions can be used as genetic markers across functional domains of well-characterized plant genes and to explore genome-wide variation (Collard and Mackill 2009). It is worthy to note that Tunisian fig germplasm represents an important genetic resource (Aljane and Ferchichi 2010) and CDDPs can efficiently mark sequences of target traits to explore, characterize and exploit this resource. Recently, the genome sequence of *Ficus carica* species was released by Mori et al. (2017) (Japanese cultivars Horraishi, about 356 Mb) and Usai et al. (2020) (cultivars Dotatto, about 333 MBbp). These sequences serve as valuable resources to verify the specificity of CDDPs used in the PCRs. Thus, the mapping process of the CDDPs primers against the whole *F. carica* genome cv Dottato (GCA_009761775.1, Usai et al. 2020) was performed. The number of in silico putative fragments ranged from 30 for MADS-4 to 58 for WRKY-R2B and ERF1 with a mean value of 46.33 putative virtual fragments. All the obtained putative fragments were higher than the amplified fragments. For instance, the number of in silico putative fragments is higher than the amplified fragments for WRKY-R2B (21), ERF1 (19) and for MADS-4 (16). This result is in accordance with the results achieved in PCR experiments, considering multiple factors that come into play during PCRs and that cannot be adequately taken into consideration into an in silico analysis (e.g. temperatures, GC bonds, primer and salt

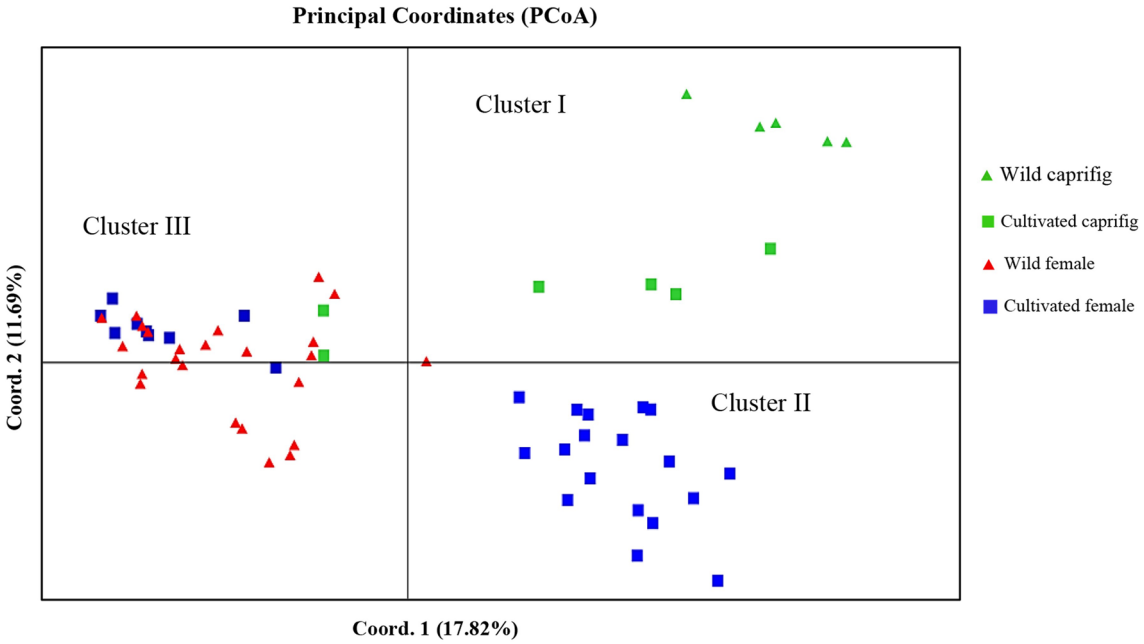

Fig. 3 Dispersion of 62 Tunisian fig accessions (*Ficus carica* L.) in the two-dimensional plane of the principal coordinate analysis (29.5% of the total diversity) based on a similarity matrix. Cluster I: includes the male figs from northern and southern Tunisia. Cluster II: assembles the cultivated female fig from the northeast, center and southwest. Cluster III: heterogeneous cluster assembles all the wild fig accessions and the remaining cultivars

Table 3 Results of analysis of molecular variance (AMOVA) for the three *Ficus carica* groups

Source variation	d.*f*	Sum of squares	Mean squares	Est. Var	Percentage of variation	*P* value
Among groups	2	318.957	159.479	6.698	19%	0.001
Within groups	59	1702.930	28.863	28.863	81%	0.001
Total	61	2021.887		35.561	100%	0.001

P value significance after 1000 random permutations

d.f. degrees of freedom, *Est. Var.* Estimated variance

concentration, etc.). We also need to consider the fact that the reference genome belongs to a different cultivar from those analyzed. Hence, the greater number of putative fragments obtained from the in silico analysis than from the PCR experiments should support the specificity of the CDDPs and the stringency of PCR conditions.

Considering CDDP as useful genetic marker, for the first time, we successfully applied this technique to analyze genetic diversity in *Ficus carica* L. genome. Thereby, the rate of molecular polymorphism obtained in the present study (TB = 204; PB = 200; PPB = 98.04%) was higher than those reported by Chatti et al. (2010) based on RAPD (PB = 60; PPB = 53.09%), ISSR (PB = 48; PPB = 54.54%), and RAMPO (PB = 63 PPB = 45.66%). As well, Dalkilic et al. (2011) revealed a low rate of polymorphism based on RAPD technique (TB = 272; PB = 76; PPB = 27.9%) in 43 different male figs from Turkey. These results proved the efficiency of CDDP markers by their capacity to reveal the polymorphism compared to non-targeted methods. In this study, the range of amplified fragments from the CDDP primers was from 9 (ABP1-2) to 24 (MYB1). Similar findings were reported by Okeh Igwe et al. (2021) for sixty-six wild and cultivated accessions of the *Musa* genus. Thus, 421 amplified fragments were detected with CDDP markers ranging from 20 (ABP1) to 51 (MYB1) per primer and MYB1 primer was the most informative displaying the highest PIC value (Okeh Igwe et al. 2021). In the current study high values of the *R*p (97.64), PIC (0.9) and PPB were found. This prove the effectiveness and the power of CDDP primer tested to survey the genetic diversity of wild and cultivated figs compared to other gel-based molecular markers including ISSR, and RAPD. Actually, CDDP markers is majorly used to assess populations purely meant for conservation and breeding purposes (Okeh Igwe et al. 2021).

The usefulness of this kind of markers has been reported in other plant species (Collard and Mackill 2009; Li et al. 2013; Jiang and Zang 2018; Saidi et al. 2018; Talebi et al. 2018; Aouadi et al. 2019).

Furthermore, CDDP targets conserved sequences of plant genes involved in adaptation such as MADS involved in controlling floral organ initiation and development, MYB implicated in abiotic and biotic stresses, ERF involved in plant disease resistance pathway, and WRKY encoding transcription factors for developmental and physiological roles (Collard and Mackill 2009). Wild and cultivated figs represent a possible plant species for study reservoir of genes related to biotic and abiotic stress. In fact, the wide distribution of the Tunisian fig trees reflects their ability to adjust to variable climatic conditions (Ben Abdelkarim et al. 2015). Ikegami et al. (2013) and Rosianski et al. (2016), reported 13 ERF genes were differentially represented in parthenocarpic and pollinated fruit inflorescence and pulp, and an intertype variation in B- and C-class MADS-box gene homologs were shown, which are supposed to be involved in sexuality. Latterly, seven markers have been investigated by amplified by Random Amplified Polymorphic DNA-Sequence Characterized amplified Region (RAPD-SCAR).As a result, only two SNP sites located on a single gene RAN1 representing the candidate for the sex-determination gene in fig (Mori et al. 2017). RAN1 is involved in the activation of ethylene receptors, which was reported to control sex phenotype in *F. carica* (Mori et al. 2017). Considering all these findings, we judged to be advantageous the choice of CDDP markers to provide additional insight into the genome of *Ficus carica* L.

The clustering analysis relatively showed the existence of a definite pattern of relationships between sex and type of the fig tree. Evidently, genotypes from the same sex and type were branching exclusively in a single or two clusters. Hence, The UPGMA analysis showed three main clusters. Cluster I includes most of the caprifigs and is organized in two main sub-clusters in accordance to the type of tree (wild or cultivated) and the geographic distribution. The second cluster II gathers most of the female fig from northeast, center and southwest of the country in one sub-cluster. Except the cultivar 'Besoul ElKhadem' branch from the other cultivars and stand alone as a sub-group. A similar situation was observed by Boudchicha et al. (2018) that characterized for the first time the genetic variability of 34 Algerian fig cultivars using 24 SSR markers. The UPGMA analysis clustered the Algerian fig accessions into two main groups. Group I includes most of the Algerian cultivars, while, group II contains only the accessions of 'Bezoul ElKhadem' cultivar (Boudchicha et al. 2018). In fact, Guillonchon (1927) and Condit (1955) described this cultivar as Tunisian varieties (Boudchicha et al. 2018).Last, cluster III consists of a mixture of wild females and all the accessions of the germplasm collection of the High Agronomic Institute of Chott Mariem. This clustering strengthens the hypothesis that a fig cultivar is defined as a collection of individuals obtained by vegetative propagation from a wild genotype that was chosen for its agronomic features and introduced into cultivation (Khadari et al. 1995; Falistocco 2009). The case of fig seems exceptional since new varieties must systematically result in the incorporation of hybrids between wild and cultivated plants (Achtak et al. 2010) which may explain this clustering. Indeed, the wild fig trees multiply through natural sexual reproduction, whereas cultivated fig trees propagate mainly with vegetative mode (Ben Abdelkrim et al. 2015). Thus, all the cultivated figs housed in Chott Mariem collection were clustered into sub-cluster (III-1) and the wild population into sub-cluster (III-2). Moreover, the presence of the two caprifig trees ('Jrani' and 'Assafri') clustered with female cultivars from Chott Mariem fig collection could reveal the existence of shared ancestry or evolutionary history and could be related to gene flow via Caprifig, the main source of pollen (Condit 1947; Giraldo et al. 2008; Aradhya et al. 2010). Since caprifigs 'Jrani' and 'Assafri' diverge from the female figs as a single group in this sub-cluster (III-1), we assume that an evident correlation between the sex of trees and the resultant clustering has occurred. This might point out that Tunisian fig may belong to two independent genetic backgrounds (male and female figs). The pattern of distribution of fig genotypes by cluster analysis revealed correspondence with the geographic regions except for some fig accessions, which formed single sub-clusters. ('Fas55' from Rafraf (Northeast) clustered with wild fig from Kerkennah Island (Center east), as well, 'Besoul ElKhadem' from Degache (Southwest), 'Hargui' from Rafraf grouped with cultivars from Kerkennah Island and 'Dhokkar' from Rafraf (Northeast) with caprifigs from Nafta (Southwest)). This is probably due to the geographical origin that has a potential influence on the genetic diversity distribution in *F. carica* germplasm collections. According to the results above, the CDDP marker systems suggested that the genetic diversity is structured dependently on the sex (Common or caprifig) and the type (wild or cultivated trees) of the fig tree and in agreement in major part with their geographical distribution. This proved the good choice of CDDP primers used that yielded unexpected results for Tunisian fig tree classification. It is noteworthy that only a few previous studies illustrated some limited clustering of fig genotypes according to geographic region (Salhi-Hannachi et al. 2006; Dalkilic et al. 2011; Essid et al. 2015; Caliskan et al. 2018). Several works reported limited genetic differentiation among fig groups from Tunisian (Chatti et al. 2007; Baraket et al. 2009, 2011; Ben Abdelkarim et al. 2015) and from over countries (Khadari et al. 2001; Giraldo et al. 2005; Aradhya et al. 2010; Caliskan et al. 2012; Ganopoulos et al. 2015; Boudchicha et al. 2018). The hypothesis that suggested a narrow genetic basis for edible figs, probably due to the common origin

of the cultivars, long history of domestication and cultivation of relatively few major cultivars, was adopted to explain level and structuration of molecular polymorphism.

The pattern of distribution of AMOVA analysis revealed that the greater part of the total genetic variation is distributed within groups (81%), whereas 19% was distributed among the groups indicating that there is a limited diversity that distinguishes Tunisian fig groups. This was displayed by the heterogeneity of the cluster III of the UPGMA and the multivariate analysis (PCoA) which gathered all the wild female and cultivated accessions housed in Chott Mariem germplasm collection. These genetic differentiations detected within the Tunisian fig groups and the low divergence scored among the groups could be explained by the occurrence of gene flow in the natural populations from which cultivars originated and the reproduction mode. Indeed, the cultivated fig trees is propagated mainly with vegetative mode, whereas wild fig trees multiply through natural sexual reproduction and seed dispersal by birds can easily facilitate the frequent exchange of genes within groups. These factors could have significant influences on the genetic variation and its partitioning (Hamrick and Godt 1996; Salhi Hannachi et al. 2005; Baraket et al. 2009; Ben Abdelkarim et al. 2015).

The efficiency of CDDPs was proved to differentiate and evaluate relationships that reflected the genetic diversity in Tunisian fig tree groups as a whole. The results also indicate that CDDP markers were very informative and can provide new insights for genetic research in *F. carica* species. In addition, in this study, we used a considerable number of fig samples displaying the different components (Wild and cultivated figs) that represent Tunisian fig germplasm, as an important fig diversity center of the Mediterranean coast of North Africa.

Conclusion

Ficus carica L. is one of the most representative species of the Mediterranean region. The current study is devoted to differentiating two compartments of *F. carica* species (Male and female) in Tunisia using Conserved DNA-derived polymorphism (CDDP). It is worthwhile to note that this is the first insight into the genome of *Ficus carica* L. with a gene target marker and discovery of genetic diversity mostly related to functional domains of plant genes. CDDP technique was a very effective and efficient tool for genetic diversity investigation and group differentiation. CDDPs suggest sex and type-dependent classification and operate consistently in agreement, with the geographic origin. Therefore, we recommend performing CDDP-based QTL mapping in future researches for flower-related, sex-related, or resistance-related traits in *Ficus carica* species. This would offer molecular mapping information for marker-assisted selection programs aimed at the improvement of multiple traits of interest. The wild *F. carica* populations represent a biological resource of extraordinary value, which can be exploited for scientific and breeding purposes. A large molecular database gathering all the revealed molecular markers characterizing the species is a prerequisite to define a core collection representing local diversity and identifying spontaneous resources reservoir of genes for resistance to biotic and abiotic stress.

Author contribution statement SH wrote the main manuscript text, executed the experiments and performed statistical analysis. GB, GU, AA, SBM and ABA checked the statistical methods, participated in the design of the tree sampling and commented on the manuscript. ASH supervised the work, provided critical feedback and corrected the manuscript.

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Declarations

Conflict of interest The authors declare that they have no conflict of interest.

References

Achtak H, Ater M, Oukabli A, Santoni S, Kjellberg F, Khadari B (2010) Traditional agroecosystems as conservatories and incubators of cultivated plant varietal diversity: the case of fig (*Ficus carica* L.) in Morocco. BMC Plant Biol 10:28

Aljane F, Ferchichi A (2009) Postharvest chemical properties and mineral contents of some fig (*Ficus carica* L.) cultivars in Tunisia. J Food Agric Environ 7(2):209–212

Aljane F, Ferchichi A (2010) Assessment of genetic diversity of Tunisian fig (*F. carica* L.) cultivars using morphological and chemical characters. Acta Bot Gall 157(1):171–182

Aljane F, Ferchichi A, Boukhris M (2008) Pomological characteristics of local fig (*Ficus carica*) cultivars in southern Tunisia. Acta Hort (ISHS) 798:123–128

Aouadi M, Guenni K, Abdallah D, Louati M, Chatti K, Baraket G, Hannachi AS (2019) Conserved DNA-derived polymorphism, new markers for genetic diversity analysis of Tunisian *Pistacia vera* L. Physiol Mol Biol Plants 25:1211–1223. https://doi.org/10.1007/s12298-019-00690-4

Aradhya MK, Stover E, Velasco D, Koehmstedt A (2010) Genetic structure and differentiation in cultivated fig (*Ficus carica* L.). Genetica 138:681–694. https://doi.org/10.1007/s10709-010-9442-3

Atia MAM, Sakr MM, Adawy SS (2017) Assessing date palm genetic diversity using different molecular markers. Methods Mol Biol 1638:125–142. https://doi.org/10.1007/978-1-4939-7159-6_12

Baraket G, Chatti K, Saddoud O, Mars M, Marrakchi M, Trifi M, Salhi Hannachi A (2009) Genetic analysis of Tunisian fig (*Ficus carica* L.) cultivars using amplified fragment length polymorphism (AFLP) markers. Sci Hortic 120:487–492

Baraket G, Chatti K, Saddoud O, Ben Abdelkrim A, Mars M, Marrakchi M, Trifi M, Salhi Hannachi A (2011) Comparative assessment of SSR and AFLP markers for evaluation of genetic diversity and conservation of fig, *Ficus carica* L., genetic resources in Tunisia. Plant Mol Biol Rep 29:171–184

Ben Abdelkrim A, Baraket G, Essalouh L, Achtak H, Khadari B, Salhi Hannachi A (2015) Use of morphological traits and microsatellite markers to characterize the Tunisian cultivated and wild figs (*Ficus carica* L.). Biochem Syst Ecol 59:209–219

Boudchicha RH, Hormaza JI, Benbouza H (2018) Diversity analysis and genetic relationships among local Algerian fig cultivars (*Ficus carica* l.) using SSR markers. S Afr J Bot 116:207–215. https://doi.org/10.1016/j.sajb.2018.03.015

Caliskan O, Polat AA (2012) Morphological diversity among fig (*Ficus carica* L.) accessions sampled from the eastern Mediterranean region of Turkey. Turk J Agric for 36:179–193

Caliskan O, Polat AA, Celikkol P, Bakir M (2012) Molecular characterization of autochthonous Turkish fig accessions. Span J Agric Res 10:130–140. https://doi.org/10.5424/sjar/2012101-094-11

Caliskan O, Bayazit S, Ilgin M, Karatas N, Ergul A (2018) Genetic diversity and population structure in caprifigs (*Ficus carica* var *caprificus*) using SSR markers. Span J Agric Res 16(3):703. https://doi.org/10.5424/sjar/2018163-11662

Chai L, Wang Z, Chai P, Chen S, Ma H (2017) Transcriptome analysis of San Pedro type fig (*Ficus carica* L.) parthenocarpic breba and non-parthenocarpic main crop reveals divergent phytohormone-related gene expression. TrEe Genet Genomes 13:83. https://doi.org/10.1007/s11295-017-1166-4

Chatti K, Saddoud O, Salhi Hannachi A, Mars M, Marrakchi M, Trifi M (2007) Analysis of genetic diversity and relationships in a Tunisian fig (*Ficus carica*) germplasm collection by random amplified microsatellite polymorphisms. J Integr Plant Biol 49(3):386–391

Chatti K, Baraket G, Abdelkrim AB, Saddoud O, Mars M, Trifi M, Hannachi AS (2010) Development of molecular tools for characterization and genetic diversity analysis in Tunisian fig (*Ficus carica*) cultivars. Biochem Genet 48(9–10):789–806

Chen Y, Li W, Hong T (2018) System optimization and genetic diversity of different color *Paeonia rockii* by CDDP markers. Acta Botan Boreali-Occiden Sin 38(10):1823–1831

Collard BC, Mackill DJ (2009) Conserved DNA-derived polymorphism (CDDP): a simple and novel method for generating DNA markers in plants. Plant Mol Biol Report 27(4):558–562

Condit IJ (1947) The Fig. The Chronica Botanica Company Waltham

Condit I (1955) Fig varieties: a monograph. Hilgardia 23:323–511

Cui Y, Wang Z, Chen S et al (2019) Proteome and transcriptome analyses reveal key molecular differences between quality parameters of commercial-ripe and tree-ripe fig (*Ficus carica* L.). BMC Plant Biol 19:146. https://doi.org/10.1186/s12870-019-1742-x

Dalkilic Z, Mestav HO, Gunver-Dalkilic G, Kocatas H (2011) Genetic diversity of male fig (*Ficus carica caprificus* L.) genotypes with random amplified polymorphic DNA (RAPD) markers. Afr J Biotec 10:519–526

Dellapor SL, Wood J, Hicks JB (1983) A plant DNA minipreparation: version II. Plant Mol Biol Rep 1:19–21

Esquinas-Alcázar J (2005) Protecting crop genetic diversity for food security: political, ethical and technical challenges. Nat Rev Genet 6:946–953. https://doi.org/10.1038/nrg1729

Essid A, Aljane F, Ferchichi A, Hormaza JI (2015) Analysis of genetic diversity of Tunisian caprifig (*Ficus carica* L.) accessions using simple sequence repeat (SSR) markers. Hereditas 152:1–7. https://doi.org/10.1186/s41065-015-0002-9

Falistocco E (2009) Presence of triploid cytotypes in the common fig (*Ficus carica* L.). Genome 52:919–925

Falistocco E (2016) Recurrent events of polyploidy in *Ficus carica* L. (Moraceae). Int J Plant Sci 177:319–325

Falistocco E (2020) The millenary history of the fig tree (*Ficus carica* L.). Adv Agric Hortic Entomol 2020(5):130

FAOSTAT (2019) Food and agriculture organization, FAO STAT database. http://www.fao.org/faostat/en/#data/QC. Accessed 27 Dec 2020

Flaishman MA, Rodov V, Stover E (2008) The fig: botany, horticulture, and breeding. Hortic Rev 34:113–196

Freiman ZE, Doronfaigenboim A, Dasmohapatra R, Yablovitz Z, Flaishman MA (2014) High-throughput sequencing analysis of common fig (*Ficus carica* L.) transcriptome during fruit ripening. Tree Genet Genomes 10(4):923–935. https://doi.org/10.1007/s11295-014-0732-2

Freiman ZE, Rosianskey Y, Dasmohapatra R, Kamara I, Flaishman MA (2015) The ambiguous ripening nature of the fig (*Ficus carica* L.) fruit: a gene expression study of potential ripening regulators and ethylene-related genes. J Exp Bot 66(11):3309. https://doi.org/10.1093/jxb/erv140

Ganopoulos I, Xanthopoulou A, Molassiotis A, Karagiannis E, Moysiadis T, Katsaris P, Aravanopoulos F, Tsaftaris A, Kalivas A, Madesis P (2015) Mediterranean basin *Ficus carica* L.: from genetic diversity and structure to authentication of a Protected Designation of Origin cultivar using microsatellite markers. Trees 29(6):1959–1971

Giraldo E, Viruel MA, Lopez-Corrales M, Hormaza JI (2005) Characterisation and cross-species transferability of microsatellites in common fig (*Ficus carica* L.). J Hortic Sci Biotechnol 80:217–224

Giraldo E, Lopez-Corrales M, Hormaza JI (2008) Optimization of the management of an ex-situ germplasm bank in common fig with SSRs. J Am Soc Hortic Sci 133(1):69–77

Groupement Interprofessionnel des fruits (GIFruits) (2019) http://gifruits.com/?p=2268&lang=fr. Accessed 4 Mar 2019

Guillonchon L (1927) La culture du figuier en Tunisie. Revue De Botanique Appliquée Et D'agriculture Coloniale 7:18–28

Hammer Ø, Harper DAT, Ryan PD (2001) PAST: paleontological statistics software package for education and data analysis. Palaeontol Electron 4:9. http://palaeo-electronica.org/2001_1/past/issue1_01.htm. Accessed 20 Oct 2021

Hamrick JL, Godt MJW (1996) Conservation genetics of endemic plant species. In: Avise JC, Hamrick JL (eds) Conservation genetics. Chapman and Hall, pp 281–304

Ikegami H, Habu T, Mori K (2013) De novo sequencing and comparative analysis of expressed sequence tags from gynodioecious fig (*Ficus carica* L.) fruits: caprifig and common fig. Tree Genet Genomes 9(4):1075–1088

Jiang L, Zang D (2018) Analysis of genetic relationships in *Rosa rugosa* using conserved DNA-derived polymorphism markers. Biotechnol Biotechnol Equip 32(1):88–94

Khadari B, Lashermes PH, Kjellberg F (1995) RAPD Fingerprints for identification and genetic characterization of fig (*Ficus carica* L.) genotypes. J Genet Breed 49:77–86

Khadari B, Hochu I, Santoni S, Kjellberg F (2001) Identification and characterization of microsatellite loci in the common fig (*Ficus carica* L.) and representative species of the genus *Ficus*. Mol Ecol 1:191–193

Kislev ME, Hartmann A, Bar-Yosef O (2006) Early domesticated fig in the Jordan Valley. Science 312:1372–1374

Knap T, Jakše J, Cregeen S, Javornik B, Hladnik M, Bandelj D (2016) Characterization and defining of a core set of novel microsatellite

markers for use in genotyping and diversity study of Adriatic fig (*Ficus carica* L.) germplasm. Braz J Bot 39:1095–1102. https://doi.org/10.1007/s40415-016-0299-2

Li T, Guo JE, Li YY, Ning H, Sun X, Zheng CS (2013) Genetic diversity assessment of chrysanthemum germplasm using conserved DNA-derived polymorphism markers. Sci Hortic 162:271–277. https://doi.org/10.1016/j.scienta.2013.08.027

Liu H, Zang F, Wu Q, Ma Y, Zheng Y, Zang D (2020) Genetic diversity and population structure of the endangered plant Salix taishanensis based on CDDP markers. Glob Ecol Conserv 24:e01242. https://doi.org/10.1016/j.gecco.2020.e01242

Marcotuli I, Mazzeo A, Colasuonno P, Terzano R, Nigro D, Porfido C, Tarantino A, Aiese Cigliano R, Sanseverino W, Gadaleta A, Ferrara G (2020) Fruit development in *Ficus carica* L.: morphological and genetic approaches to fig buds for an evolution from monoecy toward dioecy. Front Plant Sci 11:1208

Mars M (2003) Fig (*Ficus carica* L.) genetic resources and breeding. Acta Hortic 605:19–27

Mori K, Shirasawa K, Nogata H, Hirata C, Tashiro K, Habu T, Kim S, Himeno S, Kuhara S, Ikegami H (2017) Identification of RAN1 orthologue associated with sex determination through whole genome sequencing analysis in fig (*Ficus carica* L.). Sci Rep 7:46784. https://doi.org/10.1038/srep46784

Nabli M (1991) Ressources végétales en Tunisie : evaluation, valorisation et conservation. In: Rejdali M, Heywood VH (eds) Conservation des ressources végétales. Actes Editions. Institut Agronomique et Vétérinaire Hassan II, Rabat, Maroc, pp 53–58

Ochiai A (1957) Zoogeographic studies on the soleoid fishes found in Japan and its neighbouring regions. Bull Jpn Soc Sci Fish 22:526–530

Okeh Igwe D, Ihearahu Onyinye C, Adhiambo Osano A, Acquaah G, Nkem Ude G (2021) Genetic diversity and population assessment of *Musa* L. (Musaceae) employing CDDP markers. Plant Mol Biol Rep. https://doi.org/10.1007/s11105-021-01290-x

Peakall R, Smouse PE (2012) GenAlEx 6.5: genetic analysis in Excel Population genetic software for teaching and research—an update. Bioinformatics 28(19):2537–2539. https://doi.org/10.1093/bioinformatics/bts460

Poczai P, Varga I, Laos M, Cseh A, Bell N, Valkonen JP, Hyvönen J (2013) Advances in plant gene-targeted and functional markers: a review. Plant Methods 9(1):6. https://doi.org/10.1186/1746-4811-9-6

Prevost A, Wilkinson MJ (1999) A new system of comparing PCR primers applied to ISSR fingerprinting of potato cultivars. Theor Appl Genet 98(1):107–112

Rosianski Y, Doron-Faigenboim A, Freiman ZE, Lama K, Milo-Cochavi S, Dahan Y, Kerem Z, Flaishman MA (2016) Tissue-specific transcriptome and hormonal regulation of pollinated and parthenocarpic fig (*Ficus carica* L.) fruit suggest that fruit ripening is coordinated by the reproductive part of the syconium. Front Plant Sci 7:1696. https://doi.org/10.3389/fpls.2016.01696

Saidi A, Daneshvar Z, Hajibarat Z (2018) Comparison of genetic variation of Anthurium (*Anthurium andraeanum*) cultivars using SCoT, CDDP and RAPD markers. Plant Tissue Cult Biotechnol 28(2):171–182. https://doi.org/10.3329/ptcb.v28i2.39676

Salhi Hannachi A, Trifi M, Zehdi S, Mars M, Rhouma A, Marrakchi M (2004) Inter simple sequence repeat finger printings to assess genetic diversity in Tunisian fig (*Ficus carica* L.). Genet Res Crop Evol 51(3):269–275

Salhi Hannachi A, Chatti K, Mars M, Marrakchi M, Trifi M (2005) Comparative analysis of genetic diversity in two collections figs cultivars based on random amplified polymorphic DNA and inter Simple Sequence repeats fingerprints. Genet Res Crop Evol 52(5):563–573

Salhi-Hannachi A, Chatti K, Saddoud O, Mars M, Rhouma A, Marrakchi M, Trifi M (2006) Genetic diversity of different Tunisian fig (*Ficus carica* L.) collections revealed by RAPD fingerprints. Hereditas 143:15–22

Sambrook J, Fritsch EF, Maniatis T (1989) Molecular cloning, a laboratory manual, 2nd edn. Cold Spring Harbor Laboratory Press, NY, p 1659

Seyedimoradi H, Talebi R, Fayaz F (2016) Geographical diversity pattern in Iranian landrace durum wheat (*Triticum turgidum*) accessions using start codon targeted polymorphism and conserved DNA-derived polymorphism markers. Environ Exp Biol 14:63–68

Smith JSC, Chin ECL, Shu H, Smith OS, Wall SJ, Senior ML, Kresovich S, Ziegle J (1997) An evaluation of the utility of SSR loci as molecular markers in maize (*Zea mays* L.): comparisons with data from RFLPs and pedigree. Theor Appl Genet 95(1–2):163–173

Talebi R, Nosrati S, Etminan A, Mohammad Naji A (2018) Genetic diversity and population structure analysis of landrace and improved safflower (*Cartamus tinctorious* L.) germplasm using arbitrary functional gene-based molecular markers. Biotechnol Biotechnol Equip 32(5):1183–1194

Usai G, Mascagni F, Giordani T, Vangelisti A, Bosi E, Zuccolo A, Ceccarelli M, King R, Hassani-Pak K, Zambrano LS, Cavallini A (2020) Epigenetic patterns within the haplotype phased fig (*Ficus carica* L.) genome. Plant J 102(3):600–614

Vangelisti A, Zambrano LS, Caruso G et al (2019) How an ancient, salt-tolerant fruit crop, *Ficus carica* L., copes with salinity: a transcriptome analysis. Sci Rep 9:2561. https://doi.org/10.1038/s41598-019-39114-4

Wang Z, Song M, Li Y, Chen S, Ma H (2019) Differential color development and response to light deprivation of fig (*Ficus carica* L.) syconia peel and female flower tissues: transcriptome elucidation. BMC Plant Biol 19:217. https://doi.org/10.1186/s12870-019-1816-9

Wang Y, Ma Y, Jia B, Wu Q, Zang D, Yu X (2020) Analysis of the genetic diversity of the coastaland island endangered plant species *Elaeagnus macrophylla* via conserved DNA-derived polymorphism marker. PeerJ 8:8498. https://doi.org/10.7717/peerj.8498

Zambrano LS, Usai G, Vangelisti A, Mascagni F, Giordani T, Bernardi R, Cavallini A, Gucci R, Caruso G, D'Onofrio C, Quartacci MF, Picciarelli P, Conti B, Lucchi A, Natali L (2017) Cultivar-specific transcriptome prediction and annotation in *Ficus carica* L. Genom Data 13:64–66. https://doi.org/10.1016/j.gdata.2017.07.005