Morphological, Pomological, and Specific Molecular Marker Resources for Genetic Diversity Analyses in Fig (*Ficus carica* L.)

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Abstract. Fig (*Ficus carica* L.) considers the original cultivated fruit trees and currently has become extinct. Such genetic resources should be identified, documented, and conserved. Morphology, pomology, and molecular markers are successful tools in assessing genetic diversity and classifying fig accessions. Twenty-one cultivated fig (*F. carica* L.) accessions were collected from Egypt and Libya. In Egypt, fig accessions are dispersed from Sinai in the east to El-Saloom in the west and from Alexandria in the north to Aswan in the south, whereas Libyan accessions were collected from Tubryq, Benghazi, and AlKufrah. Seventeen morphological, pomological, and fruit traits were used to characterize the fig accessions. Moreover, frozen young leaves were used to extract genomic DNA; 13 expressed sequence tag (EST) common fig primer pairs with 12 intersimple sequence repeat (ISSR)-anchored primers were used to detect the genetic diversity. Analysis of variance for fig accessions showed highly significant differences concerning morphological traits, i.e., the leaf length (centimeters) and width (centimeters) ranged from 5.4 and 6 cm to 23 and 23.5 cm, for Komesrey-El-Hammam, Abodey-Giza, and Black_Mission accessions, respectively. Also, fig accessions showed different shapes of leaf edge and fruits; they were categorized into four groups: straight, waved, zigzag, and serrated. The number of leaf lobes data ranged from one lobe for the ‘Green-yellow’, ‘Sultani Red Siwa’, and ‘Sultany Red Amria’ accessions to 10 lobes in the Aswany accession. The two-way hierarchical morphological cluster analysis distributed fig accessions into two main groups. The results detected high genetic diversity for the fig accessions that could be useful in the future breeding programs. Concerning molecular data, the EST markers showed highly polymorphism and informative ($r = 0.61$; 90.0%), with a total number of identified alleles of 78. We proved that a relatively greater number of alleles per locus characterizes the targeted loci among fig accessions, for which only one and two alleles per locus have been revealed, respectively, although ISSR showed a clear pattern and bands of the primers UBC807, UBC811, UBC812, UBC814, UBC815, UBC817, UBC818, and UBC823. In conclusion, a great range of variability was detected within the fig accessions. This diversification could enrich the genetic base of this genus, and more experiments are needed to reach its full potential.

Worldwide, fig is one of the oldest traditional crops and sacred fruit trees. The common fig was one of the earliest horticultural crops to be domesticated (Mijit et al., 2017). Over the last few decades, it was discovered that many organisms have become extinct in wild environments (Presti and Wasko, 2014). This fact reproduces an accelerated loss of species that exceeds the natural rate of extinction (Primack and Rodrigues, 2006). The most critical factor of species loss is the destruction of habitat as the result of by human activity. However, human intervention is needed to ensure species survival (Volis, 2016). It has been proven that DNA-based markers are successful in assessing genetic diversity and in classifying plants (Zhou et al., 2013).

Although they are affected by environmental conditions, morphology and agronomic characteristics are helpful tools for the survival of plant species diversity. A wide range of molecular markers are used to estimate genetic polymorphism, and research on the genetic diversity of fig germplasm by Stover and Aradhya (2005), Simsek and Yildirim (2010), Podgornik et al. (2010), Dalkiliç et al. (2011), Aksoy et al. (2003), Giraldo et al. (2010), Simsek et al. (2017), and Khadivi et al. (2018) has been performed. To better conserve and use genetic resources, characterization designs of morphological variability within the collections and selection of the most significant variables should be performed carefully (Giraldo et al., 2010). Many molecular markers have been used for fig description and genetic diversity examination, such as isozymes (Cabrita et al., 2001), random amplification of polymorphic DNA (RAPD) (Akbulut et al., 2009; Dalkiliç et al., 2011; Papadopoulou et al., 2002), ISSR (Khadari et al., 2005), amplified fragment length polymorphism (Baraket et al., 2011; Cabrita et al., 2001), and simple sequence repeat (SSR) (Achtak et al., 2009; Caliskan et al., 2012), in addition to a combination of ISSR, RAPD, and SSR (Ikegami et al., 2009). Different genomic microsatellites for common fig identification have been developed by Khadari et al. (2001), Giraldo et al. (2005), and Zavodna et al. (2005).

Some techniques have been established for the detection of genetic diversity, for example, DNA polymorphism and microsatellite (Hoshino et al., 2012; Primack and Rodrigues, 2006). In addition, the microsatellite locus is distinguished by its codominant multiallelic expression, which allows for the discrimination of homozygous and heterozygous genotypes and makes the description of the different populations easier by allele frequency analysis (Bruford et al., 1996). The assessment of genetic variations during production is the principle technique for plant-breeding programs (Dean et al., 1999; Esquinas-Alcázar, 2005; Simioniu et al., 2002).

DNA fingerprinting studies in fig characterization and genetic diversity detection have been performed (Achtak et al., 2010; Akbulut et al., 2009; Aradhya et al., 2010;

- 1- Abodey-Giza
- 2- Adsey-Giza
- 3- Aswany
- 4- Barry
- 5- Bloudi
- 6- Black_Mission
- 7- Fayoumi
- 8- Green-yellow
- 9- Hamouri
- 10- Kahramany
- 11- Komesrey-EL-Hammam
- 12- Koummasri_Cairo
- 13- Mejahal
- 14- Onok_Alhamama
- 15- San_Badr
- 16- Sultani black
- 17- Sultani- Giza
- 18- Sultani Red Siwa
- 19- Sultani yellow
- 20- Sultany Red Amria
- 21- White_Fig

Fig. 1. Map of fig accessions and localities from Egypt and Libya.

Table 1. Morphological characteristics of fig accessions leaves used in the current experiment.

Accessions	Leaf length (cm)	Leaf width (cm)	Leaf edge shape	Leaf top shape	Leaf lobes no.	Leaf color	Leaf texture	Leaf neck length (cm)
Abodey-Giza	23.5 A	20.4 D	1 D	3 A	5 E	2 B	1 C	11.2 C
Adsey-Giza	14.9 K	14.5 J	2 C	3 A	7 C	2 B	1 C	7.4 J
Aswany	17.1 H	16.5 I	2 C	2 B	10 A	1 C	2 B	5.8 N
Barry	18.4 F	18.1 H	4 A	2 B	7 C	2 B	1 C	9.8 E
Bloudi	9.5 R	6.4 S	2 C	2 B	4 F	3 A	3 A	6.4 M
Black_Mission	22.2 B	23.0 A	2 C	2 B	8 B	2 B	1 C	10.1 D
Fayoumi	21.6 C	20.8 C	4 A	2 B	7 C	2 B	2 B	8.9 H
Green-yellow	16.5 I	14.0 K	2 C	2 B	1 H	2 B	2 B	9.5 F
Hamouri	11.0 Q	10.8 P	3 B	2 B	6 D	3 A	3 A	5.6 O
Kahramany	14.3 M	20.2 E	1 D	1 C	7 C	2 B	1 C	10.1 D
Komesrey-El-Hammam	5.4 S	6.0 T	3 B	2 B	3 G	2 B	2 B	3 Q
Koummasri_Cairo	20.0 E	21.2 B	3 B	2 B	8 B	1 C	3 A	11.6 A
Mejahal	14.5 L	12.0 N	4 A	2 B	3 G	3 A	2 B	6.8 L
Onok_Alhamama	12.4 O	12.5 M	4 A	3 A	5 E	3 A	3 A	8.4 I
San_Badr	18.2 G	18.5 G	3 B	3 A	3 G	2 B	2 B	11.5 B
Sultani black	13.5 N	6.8 R	2 C	3 A	5 E	3 A	2 B	6.8 L
Sultani-Giza	20.4 D	19.5 F	2 C	2 B	5 E	1 C	3 A	9.5 F
Sultani Red Siwa	14.5 L	11.0 O	4 A	3 A	1 H	3 A	2 B	4.8 P
Sultani yellow	9.5 R	6 T	2 C	2 B	5 E	3 A	2 B	6.4 M
Sultany Red Amria	15.5 J	13 L	4 A	3 A	1 H	3 A	3 A	9.4 G
White_Fig	11.4 P	8.6 Q	2 C	3 A	5 E	3 A	3 A	7.2 K
Maximum	23.5	23	4	3	10	3	3	11.6
Minimum	5.4	6	1	1	1	1	1	3
Average	15.44	14.25	2.66	2.33	5.04	2.28	2.09	8.10
Standard deviation	5.12	5.80	1.04	0.62	2.65	0.73	0.77	2.53
Sum square	876*	1248*	41.3*	13.3*	242*	20.6*	23.6*	219*

*Levels not connected by the same letter are significantly different according to least significant difference_{0.05}.

Chatti et al., 2010; Dalkiliç et al., 2011; Ikegami et al., 2009; Khadari et al., 2004). SSRs permit the study of differences between

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individual, the documentation of multiple alleles, and production of easily interpretable results with high reproducibility (Alba et al., 2009). Few experiments have been performed on the study of genetic diversity in fig germplasm around Egypt and Libya. Therefore, the purpose of the current study was to determine the genetic diversity of available fig accessions from Egypt and Libya, based on morphological and pomological characteristic, and DNA-based markers (EST and ISSR), that could be

useful for saving fig germplasm from extinction by identification, documentations, and conservation.

Materials and Methods

Experimental locations. The present study was conducted at the Agricultural Botany Department, Faculty of Agriculture, Saba Basha, Alexandria University, Egypt, and Botany and Microbiology Department,

College of Science, King Saud University, Saudi Arabia. The study was performed during the period between 2016 and 2018. In total, 21 fig accessions almost 10 years in age were collected from Egypt and Libya

(Fig. 1). Fig flowers tree cannot be seen, as they grow hidden inside the receptacle. They are pollinated by a unique wasp species from the *Agaonidae* family; *Blastophaga psenes* in the Mediterranean fig (Byng, 2014).

Morphological characteristics. Eight morphological parameters were measured to describe the fig accessions (each four replicas), such as leaf length (centimeters), leaf width (centimeters), leaf edge shape

Fig. 2. Morphological variations of fig (*Ficus carica* L.) Leaves accessions, (1) 'Kahramany', (2) 'Abodey-Giza', (3) 'Aswany', (4) 'Komesrey-El-Hammam', (5) 'Black_Mission', (6) 'Koummasri_Cairo', (7) 'Fayoumi', (8) 'San_Badr', (9) 'Barry', (10) 'Adsey-Giza', (11) 'Bioudi', (12) 'Hamouri', (13) 'White_Fig', (14) 'Sultany Red Amria', (15) 'Sultani black', (16) 'Onok_Alhamama', (17) 'Sultani Red Siwa', (18) 'Mejahal', (19) 'Green-yellow', (20) 'Sultani yellow', and (21) 'Sultani-Giza'.

Table 2. Pomological and fruit characteristics for the fig accessions.

Accessions	Fruit wt (g)	Fruit diam (mm)	Fruit length (mm)	Neck length (mm)	pH	Texture of skin	Fruit shape	Tree Vigor	Tree growth habit
Abodey-Giza	43.2	42.0	52.0	2.4	5.3	Intermediate	Globose	Medium	Erect
Adsey-Giza	29.1	31.2	41.2	3.2	5.0	Soft	Globose	Medium	Erect
Aswany	27.0	26.5	36.5	3.0	4.6	Intermediate	Globose	Medium	Weeping
Barry	41.5	44.2	40.2	2.1	6.0	Intermediate	Globose	High	Spreading
Bioudi	24.5	26.4	36.4	3.5	5.2	Intermediate	Oblate	Low	Weeping
Black_Mission	46.1	40.0	45.0	4.3	4.4	Soft	Globose	Low	Erect
Fayoumi	32.0	30.0	40.5	3.6	3.9	Soft	Globose	High	Erect
Green-yellow	33.0	24.5	34.5	5.1	4.4	Soft	Globose	High	Erect
Hamouri	27.2	20.5	30.5	4.5	5.5	Soft	Oblate	Medium	Weeping
Kahramany	27.1	20.0	30.0	4.0	5.0	Soft	Oblate	Medium	Weeping
Komesrey-El-Hammam	32.0	36.1	32.1	4.0	5.0	Soft	Globose	Medium	Spreading
Koummasri_Cairo	43.0	41.5	48.5	3.9	5.2	Soft	Globose	Medium	Erect
Mejahal	45.0	42.0	48.0	5.2	4.0	Intermediate	Globose	High	Weeping
Onok_Alhamama	47.0	42.5	48.0	4.6	4.1	Intermediate	Globose	Medium	Weeping
San_Badr	46.0	48.5	46.5	3.9	4.2	Soft	Oblate	Medium	Erect
Sultani black	33.0	36.5	46.0	5.0	4.0	Soft	Oblate	Medium	Weeping
Sultani-Giza	27.0	39.5	49.5	5.3	6.1	Intermediate	Oblate	Medium	Weeping
Sultani Red Siwa	49.0	45.0	47.0	5.0	5.6	Intermediate	Globose	High	Spreading
Sultani yellow	48.0	40.1	45.5	2.0	4.2	Soft	Globose	High	Spreading
Sultany Red Amria	34.5	33.0	43.0	3.0	3.3	Soft	Globose	High	Spreading
White_Fig	30.5	38.5	48.5	3.8	3.9	Soft	Globose	Low	Erect
Maximum	49.0	48.5	52.0	5.3	6.1	—	—	—	—
Minimum	24.5	20.0	30.0	2.0	3.3	—	—	—	—
Average	36.46	35.64	42.35	3.88	4.71	—	—	—	—
Standard deviation	8.44	8.30	6.68	1.00	0.76	—	—	—	—
Coefficient of variation	23.14	23.28	15.77	25.77	16.13	—	—	—	—

Fig. 3. Two-way hierarchical cluster analysis of 21 local fig accessions and eight morphological traits.

(1: straight, 2: waved, 3: zigzag, 4: serrated), leaf top shape (1: straight, 2: round, 3: brushes), leaf lobe number, leaf color (1: yellowish-green, 2: green, 3: dark green), leaf texture (1: smooth, 2: rough, 3: waxy rough), and leaf neck length (centimeters). Five trees and 20 leaves were selected to measure the morphological characters for each fig accession.

Pomological and fruit parameters. Nine pomological and fruit parameters were measured (each replicates five), i.e., fruit weight (grams), fruit diameter (millimeters), fruit length (millimeters), neck length (millimeters), pH, texture of skin, fruit shape, tree vigor, and growth habit. Tree growth habits are given by the fig descriptor (IPGRI and CIHEAM, 2003). Five trees were selected to measure the pomological and fruit characteristics for each fig accessions.

Molecular characterization. Young healthy leaves were collected from fig plants (≈ 2 g fresh weight). The leaves were dried in silica gel until DNA extraction. Genomic DNA was isolated from fig leaves of all accessions using CTAB according to the method of Dellaporta et al. (1983) with a slight modification.

EST polymerase chain reaction (PCR). Thirteen EST common fig primer pairs were selected, as shown in Table 3, to carry out the EST analysis. The PCR amplification reactions were performed in a 15- μ L reaction volume containing 50 ng of DNA, 7.5 μ L of iNtRON Biotech master mix (iNtRON Biotechnology, Inc., Seongnam, South Korea), and 0.2 μ moles of each primer. The EST reactions were carried out using Touchdown PCR program (New England Biolabs, Ipswich, MA). The PCR products were separated by 1.5% agarose gel electrophoresis. Previous studies on successfully used fig

Table 3. Levels of genetic information generated by 13EST primer pair sequences on 21 fig accessions.

Primer	Primers sequence (3'-5')	No. of alleles	Amplified bands				AD	He	Ho	% PIC
			Polymorphic	Monomorphic	Range (bp)	% Polymorphism				
FC 14	F: CTCATCCCTTGCTTACCTTA R: CCAAATTGCACTTGAAATAA	9	6	3	100–800	66.7	0.882	0.563	0.461	81.5
FC 21	F: GCTACACAGACAGATAGTG R: TGTGCTCGCTCTCTCTTAC	3	3	0	100–500	100	0.692	0.450	0.433	66.7
FC 22	F: GATTTCAGAGGTCATTCCAA R: CAAACTACATGGATCAAGCA	12	10	2	300–1500	83.3	0.921	0.433	0.469	90.9
FC 27	F: CTGGTCATGTGGGAAGTAGT R: ATAAATGTGGAAGGCTCAAA	5	5	0	100–300	100	0.880	0.491	0.503	80.0
Fins A1	F: AATCCCGTACTTCACTTG AGAAGTTATTGCACGGACAG	6	5	1	300–500	83.3	0.832	0.502	0.461	83.3
Fins H5	F: GACCGTATAGATGATTGGG R: CATCCTGTGAACGACACTT	5	4	1	200–300	80	0.800	0.456	0.460	80.0
Fins L12	F: GAACCTTCAACCTCAATCAA R: CTCCCTTTCTAGTCCTTA	6	5	1	300–500	83.3	0.852	0.491	0.466	83.3
Fp 21	F: AGACGAACCAGAAGACGTTA R: ATATGAACCAGCTAGGCAGT	4	3	1	300–500	75	0.789	0.499	0.465	75.0
FP 22	F: AGAATGGACTTTGAAGCTGA R: CGAAATAGGAGACGAAGTTG	6	6	0	200–500	100	0.841	0.432	0.461	83.3
FP 25	F: GAAGCGTCACATTAACTCC R: ACAAATTCGTAATGCATGAC	4	3	1	300–500	75	0.776	0.450	0.467	75.0
FP 57	F: AAATATCATCACCTGCTCAA R: GAAACGACGTAGTAAATGGC	7	5	2	300–500	71.4	0.780	0.466	0.493	79.5
Frub 29	F: CCACTTTGGAATGTCACTTGGA R: TGAACACGCCAACTGAGAATG	4	3	1	200–500	75	0.770	0.560	0.468	75.0
Frub 38	F: ACACGTGCAGTGCTGCTGA R: ACAGCTGCCCAATTCCTTGA	7	7	0	300–500	100	0.876	0.564	0.502	85.7

EST = expressed sequence tag; AD = allelic diversity; He = expected heterozygosity; Ho = observed heterozygosity; PIC = polymorphic information content.

Fig. 4. (A) Polymerase chain reaction (PCR) products patterns of 13 expressed sequence tag (EST) primers pairs used on 21 fig accessions. (B) PCR products patterns of 13 EST primers pairs used on 21 fig accessions.

microsatellite loci, as reported by Khadari et al. (2001), Giraldo et al. (2005), Ahmed et al. (2007), and Achtak et al. (2009), were used to characterize wild and cultivated fig trees.

ISSR PCR. Twelve ISSR-anchored primers were selected to carry out analysis. The PCR amplification reactions were performed in a 15- μ L reaction volume containing 50 ng of DNA, 7.5 μ L of iNtRON Biotech Taq master mix (iNtRON Biotechnology, Inc.),

and 0.25 μ moles of the primer. DNA bands of PCR product were visualized on an ultraviolet transilluminator and photographed using a professional digital camera. The polymorphic information content (PIC) was calculated by the formula $PIC = 1 - \sum x_i^2$, where x_i is the relative frequency of the i th allele. Markers were classified as informative when $PIC \geq 0.5$. (Smulders et al., 1997). Similarity coefficient matrices were calculated using the Jaccard similarity algorithm

(Jaccard, 1908) for ISSR markers, which they are dominant markers, and the simple matching algorithm for the EST, as they are co-dominant markers (Rohlf, 2000).

Statistical analysis. One-way analysis of variance in completely randomized design was used to reveal the significant differences between the fig samples. The least significant differences test was conducted to classify the significant differences among the means at (5%) level of probability.

Results and Discussion

Morphological, pomological, and fruit parameters

Analysis of variance showed highly significant differences among the fig accessions regarding morphological traits (Table 1). The smallest leaf length and width was obtained from the accession 'Komesrey-El-Hammam' (5.4 and 6 cm). The greatest value for the leaf length was recorded for 'Abodey-Giza' (23.5 cm) and 'Black_Mission' for leaf width (23 cm) (Table 1). Results recoded the lowest leaf width (centimeters) for 'Bioudi', 'Komesrey-El-Hammam', and 'Sultani yellow' (≈ 6 cm), and the greatest width was recorded for 'Black_Mission' (23 cm). Highly significant variations were obtained between the different fig accessions, as shown in Table 1.

The fig accessions provided different shapes of leaf edges and fruits; they were categorized into four groups (Fig. 2), including accessions 'Abodey-Giza' and 'Kahramany' as straight (1); waved (2), which included 'Adsey-Giza', 'Sultani black', 'Aswany', 'Bioudi', 'Green-yellow', 'Sultani-Giza', 'Black_Mission', 'Sultani yellow', and 'White_Fig'; zigzag (3), which included 'Hamouri', 'Komesrey-El-Hammam', 'Koummasri_Cairo', 'San_Badr'; and serrated (4), which included 'Barry', 'Fayoumi', 'Mejahal', 'Onok_Alhamama', 'Sultani Red Siwa', and 'Sultany Red Amria' (Table 1). Leaf top shape was divided into three groups: straight (1), which included 'Kahramany'; round (2), which included 'Aswany', 'Barry', 'Bioudi', 'Black_Mission', 'Fayoumi', 'Green-yellow', 'Hamouri', 'Komesrey-El-Hammam', 'Koummasri_Cairo', 'Mejahal', 'Sultani-Giza', and 'Sultani yellow'; and brushes (3), which have one accession, 'Abodey-Giza', 'Adsey-Giza', 'Onok_Alhamama', 'San_Badr', 'Sultani black', 'Sultani Red Siwa', 'Sultany Red Amria', and 'White_Fig' (Table 1).

Leaf lobes number ranged from one lobe for the accessions 'Green-yellow', 'Sultani Red Siwa', and 'Sultany Red Amria'; three lobes for 'Komesrey-El-Hammam', 'Mejahal', and 'San_Badr'; four lobes for 'Bioudi'; five lobes 'Abodey-Giza', 'Onok_Alhamama', 'Sultani black', 'Sultani-Giza', 'Sultani yellow', and 'White_Fig'; six lobes for 'Hamouri'; seven lobes for 'Adsey-Giza', 'Barry', 'Fayoumi', and 'Kahramany'; eight lobes for 'Black_Mission' and 'Koummasri_Cairo'; and 10 lobes 'Aswany' (Fig. 2; Table 1). The leaf color of the fig accessions arranged from yellowish-green color for the

Fig. 4. (Continued)

accessions, i.e., 'Aswany', 'Sultani-Giza', green for 'Abodey-Giza', 'Adsey-Giza', 'Barry', 'Black_Mission', 'Fayoumi', 'Green-yellow', 'Kahramany', 'Komesrey-El-Hammam', and 'San_Badr' and dark green for 'Bioudi', 'Hamouri', 'Mejahal', 'Onok_Alhamama', 'Sultani black', 'Sultani Red Siwa', 'Sultani yellow', 'Sultani Red Amria', and 'White_Fig' (Fig. 2; Table 1).

The leaf texture detected for the fig accessions 'Aswany', 'Fayoumi', 'Green-yellow', 'Komesrey-El-Hammam', 'Mejahal', 'Sultani yellow', and 'Sultani black' was recorded as rough. The leaf texture in the accessions 'Abodey-Giza', 'Adsey-Giza', 'Barry', 'Black_Mission', and 'Kahramany' was recorded as waxy-smooth, and the other accessions were waxy rough (Fig. 2; Table 1). It could be concluded that there is a wide range of variability within the cultivated fig accessions for the leaf texture.

The tallest leaf neck was recorded for 'Kommasri-Cairo' at 11.6 cm, whereas the lowest recorded for 'Kommasri-El-Hammam', on average, was 3 cm, as shown in Table 1. Fruit weight (grams) is the main pomological characteristic that differentiates the studied fig accessions. 'Sultani Red Siwa' fig recorded the greatest fruit weight (grams),

and the maximum value was 49.0 g, whereas the minimum value was 24.5 g, recorded for the 'Bioudi' accession; the average value was 36.46 g (Table 2). Fruit diameter (millimeters) showed an average 35.64 mm, and the greatest value was 48.5 mm compared with the lowest value of 20 mm, found in 'San_Badr' and 'Kahramany', respectively. For fruit length (millimeters) and neck length (mm), data in Table 2 show that the maximum values were 52 mm and 5.3 mm for 'Abodey-Giza' and 'Sultani-Giza', whereas the lowest values were 30 mm and 2 mm, recorded for 'Kahramany' and 'Sultani' yellow accessions, with an average of 42.35 and 3.88 mm, respectively (Table 2). The pH value ranged from 3.3 to 6.1 for all fig accessions; the average was 4.71. The texture of skin differed from soft to intermediate, whereas the fruit shape was globose and oblate, as shown in Table 2.

Data showed a wide range of variability within the cultivated fig accessions. Morphological data will be useful in characterization and creating the first reference and catalogue of fig accessions. Our results are in agreement with Ahmed et al. (2015), who used eight morphological parameters and 17 SSR loci to characterize 71 cultivated and wild Tunisian

fig trees. In addition, Stover and Aradhya (2005) determined two accessions to be first crops and the other 74 accessions were main crops. Our results are in agreement with Çalışkan and Polat (2008), who studied the fruit characteristics of fig cultivars and genotypes grown in Turkey. They indicated extensive diversity among the Turkish figs based on fresh fig traits. In addition, our current study is in agreement with Polat and Özkaya (2005), who studied plant and fruit characteristics of fig genotypes.

Two-way hierarchical morphological cluster analysis

Morphological characters were determined by the two-way hierarchical cluster analysis of the 21 fig accessions using JMP 7.0 software [SAS Institute, Cary, NC (SAS Institute, 1989)]. In the first round of hierarchical clustering, the fig accessions were distributed into two main groups. The first group included five clusters separated into two clusters. The first cluster included accessions 'Sultani Giza', 'Kommasri Cairo', the next cluster included 'San-Badr' and 'Green-yellow', the third cluster contained 'Adsey-Giza', and 'Aswany', the fourth cluster consisted of 'Fayoumi and Barry', and the fifth cluster included 'Abodey-Giza', 'Black_Mission', and 'Kahramany' (Fig. 3). In the next wave of clusters, the eight leaf morphological traits were distributed into two clusters. The first cluster consisted of the traits 'Kommasry-Elhammam', 'Bioudi', 'Sultani yellow', 'Hamouri', 'White Fig', and 'Sultani-Black'. In contrast, the other cluster included the traits 'Onok-El-Hamama', 'Sultani Red Amria', 'Mejahal', and 'Sultani Red Siwa' (Fig. 3).

Our results are similar with other reports in of fig collections from different countries, i.e., Tunisia (Chatti et al., 2003), Turkey (Çalışkan and Polat, 2008), Morocco (Oukabli et al., 2002), Spain (Sanches et al., 2002), Lebanon (Chalack et al., 2005), and Jordan (Almajalia et al., 2012), which reported high diversity in pomological and leaf-related traits, and these could be useful as an efficient marker system to discriminate between fig genotypes.

Molecular analyses

EST analysis. In total, 13 primers of EST markers specific for genes were used to study the genetic relatedness of 21 fig accessions (Table 3; Fig. 4A and B). The EST patterns were in general highly polymorphic and informative. Some primers pairs showed mono-alleles, such as FC21, FinsA1, FinsL12, and FP21, whereas other primer pairs produced two alleles, such as FC22, FC27, FinsH5, and Frub29. The other EST primers pairs gave multialleles in their morpho-pattern. In the present investigation, we proved that a relatively greater number of alleles per locus characterizes the targeted loci among 21 fig accessions, for which only one and two alleles per locus have been revealed, respectively. Moreover, the total number of identified alleles was 78 in the 21 fig accessions. The lowest

Fig. 5. Dendrogram based on simple matching similarity coefficient of 21 fig accessions generated using expressed sequence tag molecular markers.

value was recorded for FC21 primer pairs and the greatest value was recorded for four EST primer pairs such as FC22 (12) (Table 3).

Polymorphic percentage (PIC %) values were positively correlated ($r = 0.61$) with the number of amplified alleles per primer. The high average PIC value of 90.9 indicated the presence of low genetic similarity among 21 fig accessions, and the 13 primer pairs of EST markers were effective for the characterization of fig accessions. Our study, by using EST markers, revealed a high level of genetic diversity among fig accessions. Polymorphism between genotypes can arise through nucleotide changes that prevent amplification by introducing a mismatch at one priming site, deletion of a priming site, insertions that render priming sites too distant to support amplification, and insertions or deletions that change the size of the amplified product 21.

Depending on the primer pair and the DNA sample, a total of 78 bands were observed, with six bands per primer pair. Of these 78 amplified fragments, 2.25% were monomorphic whereas 97.75% were polymorphic among the 21 fig accessions. Of the 13 EST primer pairs, four revealed approximately 100% polymorphism: FC21, FC27, FP22, and Frub 38.

EST dendrogram. The EST dendrogram presents clusters that are divided into two main clusters by 65% genetic similarity, as shown in Fig. 5. The first cluster includes three fig accessions by 74% similarity with 'Aswany', 'Kommasri-Cairo', and 'San Bader', whereas the next cluster divided into two subclusters with 74%. The first subcluster includes 'Sultani Cairo' and 'Kommasri-El-Hammam', with 76% genetic similarity. Finally, the last cluster divided into other two subclusters by 75% genetic similarity. The first one includes (79%) 'Adassi', 'Kahramany', 'Fayoumi', 'Sultani-Red-Siwa', 'Black-Mission', 'Barry', 'Hamouri', 'Onok-El-Hamama', and 'Mejahal'. The next one (75%) includes 'Aboudi', 'Bioudi', 'Sultany-Red-Amria', 'Akhdar Safrawi', 'Sultani Black', 'Sultany Yellow', 'White Fig', 'Kommasri-Cairo', and 'San-Badr'. All clusters confirmed seven groups; each group contained some fig accessions from the basic 21 fig accessions, and the genetic distance ranged between 0.65% and 0.90%.

ISSR analysis. ISSR patterns are presented in Fig. 6A and B. The PCR products of 12 ISSR-anchored primers were separated by 1.5% agarose gel electrophoresis, photographed, and scored for analysis. The pattern

showed genetic diversity among the used genotypes and different common bands were amplified from different primers. Clear pattern and bands were observed in the pattern of the primers UBC807, UBC811, UBC812, and UBC814 (Table 4) and for the primers UBC815, UBC817, UBC818, and UBC823. The other primers showed faint bands, which could be related to nonspecificity or inadequate handling.

ISSR dendrogram. The ISSR dendrogram (Fig. 7) presents clusters that were divided into subclusters that confirmed 10 groups; each group contained some fig accessions from the basic 21 fig accessions and the genetic distance ranged between 0.70 and 0.93. The cluster divided into two main groups with 71% genetic similarity, the first one contained two fig accessions, namely 'Sultani-Red-Siwa' and 'Sultani-Black', with 88% similarity, whereas the other group was divided into many different groups. The first one was contained 'Sultani-Cairo' and 'Fayoumi' (85%). The next was 'Adassi', 'Aboudi', and 'Aswany' (83%). The third was 'Kommasri-Cairo' and 'Black-Mission'. The fourth was 'San-Badr' (88%). The fifth was 'Barry', 'White-Fig', 'Onok-El-Hamama' and 'Sultani-Red-Amria' (86%). The sixth was 'Bioudi' (83%). The seventh was 'Hamouri' and 'Sultani-yellow' (78%). The eighth was 'Kommasri-El-Hammam' and 'Akhdar-Safrawi' (82%). The ninth was 'Kahramany and Mejahal' (79%) (Fig. 7).

For the fig accessions, the observed heterozygosity were similar to the expected heterozygosity for most of the primer pairs tested (Tables 3 and 4) in addition, allelic diversity (AD) showed highly significant variations between all primers for the 21 fig accessions. With EST primers, the results of the PIC analysis, the maximum AD was obtained for marker FC22 (0.921), and its value was greater than those found by Aradhya et al. (2010) and Chatti et al. (2010). In addition, the lowest AD occurred in the marker with the smallest PIC value (FC21; 0. 0.692). In contrast, with ISSR primer, the maximum AD was obtained for marker UPC 810 (0.961) and the lowest value was UPS 808 (0. 0.683).

These results were similar to those of Saddoud et al. (2011), who characterized 18 Tunisian fig accessions by using SSR markers to make a comparison between the genetic polymorphism with the observed phenotypic variation by employing six microsatellite primers, 39 alleles, and 59 genotypes. In addition, our study was in agreement with that of Khadari et al. (2001), who found eight primer pairs produced amplification products by designed 20 microsatellites to fig characterization and polymorphic in 14 fig accessions and two French wild-growing populations of *F. carica*.

Furthermore, our results of 21 fig accessions are in line with Çalışkan and Polat (2008), who used the analysis of 10 SSR loci to study the genetic variabilities of 76 Turkish fig accessions from the Hatay Province.

Fig. 6. (A) Polymerase chain reaction (PCR) products patterns of 12 intersimple sequence repeat (ISSR) primers pairs used on 21 fig accessions. (B) PCR products patterns of 12 ISSR primers pairs used on 21 fig accessions.

At the same time, Ahmed et al. (2015) and Sarwar and Qaiser (2012) showed that SSR loci are sufficient to implement in the study of fig germplasm. Previous studies reported the homonymy, mislabeling, and synonymy problems in fig genotypes worldwide, i.e., Khadari et al. (2005), also, Dalkiliç et al. (2011) used RAPD markers to determine three mislabeled and four homonym genotypes among 43 Turkish figs. In addition, Essid et al. (2015) detected three unremarkable accessions due to synonyms and analyzed several homonyms within 20 Tunisian figs. In contrast, Aradhya et al. (2010) reported that fig accessions from Turkmenistan

are genetically different from the Mediterranean and the Caucasus figs. In current research, 87 bands were detected (average six bands/primer). Of these 78 amplified fragments, 2.25% were monomorphic, whereas 97.75% were polymorphic among the 21 fig accessions. Of the 13 EST primer pairs, four revealed approximately 100% polymorphism: FC21, FC27, FP22, and Frub 38. These results are similar to those of Haddou et al. (2018), who studied molecular characterization of the Moroccan fig. They detected by seven ISSR primers and nine loci SSR 54 (average of 8 per primers) and 42 alleles (five alleles per locus). In our results, the number

of alleles per loci identified was greater than that obtained by Giraldo et al. (2008) and lower than that reported by Khadari et al. (2005). These parameters indicated the existence of genetic polymorphism between the genotypes evaluated in this study reached 100%. These results are in agreement with other studies conducted by Chatti et al. (2004) (71.6%), Aljane and Ferchichi (2009a) (70.7%), and Gaaliche et al. (2012) (51.9%).

The current research is in agreement with Ali-Shtayeh et al. (2014), who used 22 landrace (*Ficus carica* L. sativa) and two wild forms to study the differentiation and genetic diversity with related relationships in Palestine via PCR-based RAPD and pomological markers. They reported that both pomological and RAPD markers are useful tools for elucidating in part denomination problems and relationships among fig cultivars. SSR markers are commonly used for the calculation of the diversity of fig trees (Achtak et al., 2009, 2010; Giraldo et al., 2008; Khadari et al., 2004; Saddoud et al., 2007).

Today, SSRs are mostly the markers of choice for many breeding programs (Hernandez, 2005), because of their codominant nature and intraspecific polymorphism (highly informative), in addition to their easy automated detection by PCR.

The current results are similar with other study in Tunisia by Gaaliche et al. (2012), who focused on the fig germplasm characterization of 17 cultivars based on morphological and pomological traits. Their results revealed a large variability within the local fig germplasms. They reported that the diversity is currently threatened by genetic erosion. Measure of conservation is necessary to be undertaken. Furthermore, in recent years previous studies have developed genomic microsatellite markers for common fig tree (Achtak et al., 2009; Giraldo et al., 2005; Khadari et al., 2001).

In other research, phenotypic and DNA-based markers used for identification, documentation and characterization of fruit crops, figs, and others (Abdelsalam et al., 2018; Agarwal et al., 2008; Aljane and Ferchichi, 2009b; Almajali et al., 2012; Baraket et al., 2011; Gaaliche et al., 2012; Khadari et al., 2003; Saddar and Atteyyeh, 2006). DNA-based markers methods have proven to be powerful tools to measure genetic diversity in figs. In contrast to morphological markers, molecular markers are stable and are not confounded by environmental effects (Achtak et al., 2009; Saddar and Atteyyeh, 2006).

Conclusions

The main goals of this study were to describe and identify fig accessions by setting a series of morphological, pomological and fruit characters using all the available traits. Genetic diversity of the fig accessions in Egypt and Libya by depend on specific molecular techniques such as EST and

ISSR-PCR were investigated. The results showed that based on previous parameters there are highly genetic diversity among the fig accessions could be useful in future breeding programs. These differentiations

could enrich the genetic base of fig accessions and required more studies to achieve the maximum usefulness from this diversity because there are no clear breeding programs in developmental countries to characterize or

improve the fig accessions. With recommendation, further analysis should be performed before stating that the study showed high genetic diversity between the fig accessions and could be useful in breeding programs.

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Fig. 6. (Continued)

Table 4. Levels of genetic information generated by 12 ISSR primer pair sequences on 21 fig accessions.

Primer	Sequence (3'-5')	Amplified bands							% PIC	
		No. of alleles	Polymorphic	Monomorphic	Range (bp)	% polymorphism	AD	He		Ho
UBC 807	(AG) ₈ T	12	6	6	200–1000	50.0	0.831	0.432	0.533	81.5
UBC 808	(AG) ₈ C	5	4	1	500–1000	80.0	0.683	0.388	0.418	66.7
UBC 810	(GA) ₈ T	5	4	1	200–100	80.0	0.961	0.512	0.610	90.9
UBC 811	(GA) ₈ C	6	5	1	200–1000	83.3	0.881	0.459	0.550	80.0
UBC 812	(GA) ₈ A	7	5	2	300–1000	71.4	0.851	0.440	0.540	83.3
UBC 814	(CT) ₈ A	5	4	1	200–500	80.0	0.821	0.432	0.533	80.0
UBC 815	(CT) ₈ G	9	7	2	500–1000	77.7	0.872	0.560	0.566	83.3
UBC 816	(CA) ₈ T	6	4	2	500–1000	66.6	0.772	0.389	0.483	75.0
UBC 817	(CA) ₈ A	6	6	0	300–1000	100	0.843	0.501	0.533	83.3
UBC 818	(CA) ₈ G	7	5	2	200–800	71.4	0.794	0.447	0.347	75.0
UBC 823	(TC) ₈ C	13	8	5	500–1000	61.5	0.810	0.430	0.531	79.5
UBC 861	(ACC) ₆	8	6	2	300–1000	75.0	0.783	0.342	0.441	75.0

ISSR = intersimple sequence repeat; AD = allelic diversity; He = expected heterozygosity; Ho = observed heterozygosity; PIC = polymorphic information content.

Fig. 7. Dendrogram based on simple matching similarity coefficient of 21 fig accessions generated using intersimple sequence repeat molecular markers.

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