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Evaluation of genetic diversity among Jordanian fig germplasm accessions by morphological traits and ISSR markers

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ABSTRACT

This study describes phenotypic variation in leaf and fruit traits and investigates genetic relationships among 24 landrace (*Ficus carica* L. *sativa*) and 6 wild form (*F. carica* L. *caprificus*) accessions of fig using inter simple sequences repeat (ISSR) markers. For phenotypic traits, pair-wise Euclidean distances ranged from 0.90 to 9.80. Principal component analysis revealed that the first five components explained 74.14% of the total morphological variation, where fruit traits contributed most of the total variation. Nei's genetic distance based on ISSR data ranged from 0.00 to 0.83, suggesting that the collected accessions are genetically diverse. UPGMA clustering based on phenotypic traits compared with that based on ISSR data were not consistent, however, some common groupings were observed in ISSR and phenotypic traits. In most cases, accessions collected from the same landrace or from fig wild forms tended to cluster together, confirming that names given by farmers to the collected landrace accessions are consistent and confirming also the common genetic background of wild fig accessions. High phenotypic and ISSR variability indicate that fig collections used in this study include rich and valuable genes for fig breeding. ISSR screening revealed the presence of the same ISSR allelic profile for accessions from the same landrace or wild form accessions, indicating that ISSR can provide the basic information necessary to help gene banks to conserve materials from different genetic background rather than duplicates from same clone.

1. Introduction

Fig (*Ficus carica* L.) is one of the oldest traditional crops and sacred fruit tree widely present in several countries around the Mediterranean basin (Condit, 1947; El-Rayes, 2000). There are two subspecies of fig: *F. carica* L. *sativa* (common fig) and *F. carica* L. *caprificus* (caprifig or wild type) (Edmond et al., 1975; Weiblen, 2000). A detailed knowledge of the amount of genetic variability among existing fig germplasm is pursued to broaden the genetic base of fig breeding programs (Morton et al., 1987; Oukabli et al., 2002; Papadopoulou et al., 2002; Chatti et al., 2003; Ateyyeh and Sadder, 2006). Locally adapted fig landraces and their wild forms, primarily used for fresh consumption, can be found in Jordan with high level phenotypic variation in fruit colors, sizes, shapes, and flavors. Local fig landraces are available with different local Jordanian names which are mainly given based on fruit shape and fruit skin color; the famous local names are 'Zrakee', 'Kortomanee', 'Ajlounee', 'Esalee', 'Mwazee', 'Swadee', 'Kdaree' and 'Byadee' (Shdiefat, 2007).

During the past decades many constraints combine to limit and to reduce the growing areas of fig in Jordan, which led to a strong genetic decline of the local gene pool with the real risk of useful genetic resources for future breeding programs. In Jordan, the cultivated area with fig trees is sharply decreased from 1085 ha in 1991 to 542 ha in 2007 (Agricultural Statistics, 2006). The reduction in fig cultivated area in Jordan is mainly due to frequent drought seasons, the reduction of the number of genotypes selected and maintained since ancient time, intensive urbanization and the replacement of fig with olive trees (Salhi-Hannachi et al., 2004). To safeguard the remaining fig germplasm, it is necessary to preserve the present genetic resources as much as possible, not only for the long term survival of the species but also to ensure enough variability for future breeding programs (Esquinas-Alcazar, 2005).

Cultivars identification and evaluation of genetic resources in fig are based mainly on morphopomological characterization (Oukabli et al., 2002), however relying only on morphopomological traits in cultivars identification might have some limitations: (i) the time from planting to first fruiting is too long, ranging from 5 to 7 years, (ii) there is no reliable way to distinguish cultivars at early stage of fig tree growth due to similar morphological characteristics of the early growing cuttings and (iii) morphological traits would be influenced by environmental factors (Condit,

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1955). Therefore, DNA-based markers are considered as useful tools for identification of plant cultivars (Gilbert et al., 1999; Khadari et al., 2001; Lai et al., 1998; Guasmi et al., 2006), however, morphological characterization is a highly recommended as a first step before starting DNA-based studies (Hoogendijk and Williams, 2001). Molecular marker methods based on the Polymerase Chain Reaction (PCR) technique could serve to differentiate clones and varieties according to DNA fragment lengths. Molecular techniques as restriction fragment length polymorphisms (RFLPs) of the total genome, random amplified polymorphic DNA (RAPD), amplified fragment length polymorphisms (AFLPs), inter simple sequence repeats (ISSR) and simple sequence repeats (SSR) have been successfully used for characterizing fig germplasm (Khadari et al., 1995, 2001, 2003a,b,c; Galderisi et al., 1999; Korbin et al., 2002; Resta et al., 2003; Amel et al., 2004, 2006; Salhi-Hannachi et al., 2006; Guasmi et al., 2006; Sadder and Ateyyeh, 2006; Dalkiliç et al., 2011). Inter-single sequence repeats (ISSR) is widely used technique in fig genetic diversity studies (Moreno et al., 1998) and genetic mapping (Bornet and Branched, 2001). ISSR markers have been reported to be as efficient as SSR (Khadari et al., 2001, 2003a,b,c; Ikegami et al., 2009) and RAPD markers (Khadari et al., 1995; Papadopoulou et al., 2002; Ruan et al., 2004) in fig genetic analysis. During recent years, the use of ISSR markers for resolving the genetic diversity in fig trees were remarkable (Khadari et al., 2003c, 2005; Amel et al., 2004; Ikegami et al., 2009).

Unfortunately, there has been little research dealing with the genetic diversity in fig germplasm. The present research is an extension of previous works (Ateyyeh and Sadder, 2006; Sadder and Ateyyeh, 2006) on Jordanian fig germplasm. Our objectives were to assess the genetic diversity in seven different fig landraces and six fig wild form accessions from Jordan using morphological traits and ISSR markers and to compare the discriminating power of morphological- and ISSR-based markers to differentiate between Jordanian common fig collections.

2. Materials and method

2.1. Plant materials

In January 1999, field trips were organized by the National Center for Agricultural Research and Extension (NCARE) to collect stem cuttings from fig landrace varieties according to their local names from different areas in Jordan (Table 1). In total, seven fig landraces were localized, namely; 'Mwazee' (Mw), 'Kortomanee' (Kr), 'Zrakee' (Zr), 'Ajlounee' (Aj), 'Kdaree' (Kd), 'Byadee' (By) and 'Hmaree' (Hm). Thereafter, stem cuttings (2–4 accessions/landrace; 24 accessions in total) were collected and subsequently rooted and transplanted to field to be conserved at the NCARE, Al-Mshaqer agricultural station (semi-arid, 32°36′ N, 35°53′ E and 790 m above sea level). Additionally, six wild trees (W1, W2, W3, W4, W5 and W6) were localized and labeled in March, 2007 in Wadi Al-Karak area, Karak, Jordan (semi-arid, 31°20′ N, 35°65′ E and 625 m above sea level). Thirty one morphological traits including nine quantitative and 22 qualitative traits, and five ISSR were used to describe and compare the diversity observed in fig landraces and fig wild forms from Jordan.

2.2. Morphological traits

Fig landrace and fig wild form trees (thereafter called accessions) were characterized for morphological traits according to the guidelines provided by the International Plant Genetic Resources Institute (IPGRI-FAO, 2003) in the 'Descriptors of fig (*F. carica* and related *Ficus* spp.)'. Pomological characteristics were determined from mature summer crop fruits in July and August, 2007. All leaf related traits were recorded from the fifth fully expanded leaves. Quantitative traits included leaf length and width (cm), leaf area (cm^2), length of leaf stalk/length of leaf, petiole length (mm), fruit weight (g), fruit width and length (cm) and fruit flesh thickness (mm), by recording data from 10 randomly selected fruits or leaves from each accession. Qualitative traits included nine leaf related-traits: leaf shape, shape of leaf lobes, shape of leaf base, number of lobes per leaf, location of little lateral lobes, leaf margin dentation, leaf margin type, leaf color and petiole color, and thirteen morpho-pomological traits: fruit apex shape, shape of fruit stalk, fruit symmetry, uniformity of fruit size, easy of peeling, fruit skin crack, color of scales around ostiolum, resistance to ostiole end-cracks, fruit skin ground color, fruit lenticels quantity and their color and pulp flavor and color.

2.3. ISSR analysis

Fresh leaves were collected from fig accessions and directly dried using liquid nitrogen. Genomic DNA was isolated from the collected leaves using Cetyl Trimethyl Ammonium Bromide (CTAB) method according to Doyle and Doyle (1990). The concentration and purity of extracted DNA were assessed by spectrophotometer (Perkin Elmer, Lambda EZ201, USA), then diluted to 50 ng/ml for PCR amplification. A set of eight ISSR primers developed by Khadari et al. (2005) was used for screening in the current study already reported to be polymorphic on fig (Table 2). The ISSR primers used were IMA5, IMA9, UBC841, IMA12, UBC818, IMA8, IMA834 and IMA303. Initial screening was done using all available primers using DNA from eight accessions; 7 accessions representing local fig landraces and one from fig wild forms. Only five ISSR primers (IMA5, UBC 841, IMA12, UBC818 and IMA834) that reproduced consistently across successive PCR reactions were included in the analysis.

PCR conditions were optimized using different concentrations of template DNA and Mg^{2+} as well as different annealing temperatures. Based on the optimization experiments, the PCR was carried out in 15 μl final volume as follows: 3 μl (50 ng DNA μl^{-1}), 1.5 μl 10× PCR buffer, 0.5 μl of each primer (100 mM), 1.5 μl from dNTPs (10 mM), 0.4 μl of Taq polymerase (0.75 unit), 1 μl $MgCl_2$ (25 mM) and 7.1 μl of deionized distilled H_2O. PCR reactions were carried out in Peltier Thermal Cycler (PTC-200, MJ-Research, USA) programmed for an initial denaturation step of 95 °C for 4 min, followed by 45 cycles of 94 °C for 1 min, 48–49 °C for 1 min, 72 °C for 1.5 min and a final extension at 72 °C for 10 min. Amplified products were separated by electrophoresis in 1.5% (w/v) agarose gels (Biobasic, Canada) in TBE buffer (Tris–boric acid–EDTA), visualized by ethidium bromide staining and photographed under UV light with a Gel Doc system (UVP, Bio Doc, Upland, USA). Each sample was amplified at least twice to verify reproducibility.

2.4. Statistical analysis

For the 9 quantitative traits, descriptive statistics were computed for each landrace and fig wild accessions using the statistical package for social studies software (SPSS Inc., 2007). Analysis of variance (ANOVA) was carried out for the 9 selected quantitative traits. Qualitative data was described as discrete variables for each accession. To avoid the effects due to scaling differences, mean of each character was normalized prior to cluster analyses using Z-scores. Thereafter, Euclidean distance coefficient for pairs of entries (i.e. accessions) was computed using NTSYSY-pc (Numerical Taxonomy and Multivariate Analysis for personal computer) software program version 2.00 (Rohlf, 1998). To better understand the patterns of variation among accessions/genotypes, distance matrix generated from morphological data was used as input data for cluster analysis based on unweighted pair-group method of arithmetic average (UPGMA), and to compute principle component analysis

Table 1
Eco-geographical information about Jordanian fig landraces and fig wild forms collection sites.

Village	Genotype	Genotype abbreviation	Number of clones	Latitude (north)	Longitude (east)	Elevation (m)	Average rainfall (mm)
Abu Nsaier	Mwazee	Mw	3	32°52′	35°52′	662	400
Ain Al-Basha	Kortomanee	Kr	4	32°34′	35°50′	668	500
	Ajlounee	Aj	4				
	Byadee	By	4				
Abu Nsaier (Village)	Zrakee	Zr	2	32°31′	35°52′	972	450
Wadi Al- Sair	Kdaree	KD	4	31°50′	35°50′	907	500
Zayy	Hmaree	HM	3	32°53′	35°43′	919	500
Wade Al-Karak	Wild forms	W	6	31°20′	35°65′	625	350

Table 2
List of ISSR-PCR primers, their sequences and the degree of polymorphism obtained among over 24 landrace accessions and 6 fig wild forms.

Primer	Sequence (5′–3′)	Annealing temperature	No. of total amplified markers	No. of polymorphic markers	Percentage of polymorphic markers (PPB)%	PIC value	Range of the bands size (bp)
IMA5	CACACACACACACAGT	48 °C	3	3	100	0.193	373–1238
IMA9	GAGAGAGAGAGAGACG	–	–	–	–	–	–
UBC 841	GAGAGAGAGAGAGACTCAG	48 °C	5	5	100	0.25	487–1366
IMA12	CACACACACACACATG	49 °C	4	3	75	0.30	652–1300
UBC818	CACACACACACACAG	48 °C	4	4	100	0.27	250–833
IMA8	GAGAGAGAGAGAGAGT	–	–	–	–	–	–
IMA834	AGAGAGAGAGAGAGCTT	48 °C	6	5	83	0.28	300–1230
IMA303	(AGT)(AGT)(AGT) CACCACCACCACCAC	–	–	–	–	–	–

Table 3
Mean, maximum, minimum, ranges and coefficient of variation (CV) for 9 quantitative traits recorded over 24 landrace accessions and 6 fig wild forms.

Trait	Min.	Mean	Max.	Range	CV%	*F*-value
Leaf length	10.66	17.90	27.10	16.44	25.14	**
Leaf width	10.90	15.90	20.60	9.70	16.35	*
Leaf area	117.30	293.60	528.50	411.20	38.83	**
Length of leaf stalk/Leaf length	0.39	1.90	3.25	2.86	26.32	**
Petiole length	5.24	61.10	95.00	89.76	31.92	**
Fruit weight	3.50	19.20	41.97	38.47	51.04	*
Fruit width	2.08	3.70	5.18	3.10	24.32	**
Fruit length	2.45	3.70	5.16	2.71	18.91	**
Fruit flesh thickness	7.00	14.90	21.8	14.80	23.35	**

* Significant at the 0.05 probability levels.
** Significant at the 0.01 probability levels.

(PCA). For each factor, loading value above 0.5 considered as significant.

For ISSR data analysis, amplification products were scored as "present" or "absent" and transferred to a binary code with 1 or 0, respectively. The polymorphic information content (PIC) for each selected ISSR primer was calculated with formula described by Roláand-Ruiz et al. (2000): $PIC_i = 2f_i\ (1 - f_i)$, where PIC_i is the polymorphic information content of primer i, f_i the frequency of the marker bands which were present, and $1 - f_i$ the frequency of the marker bands were absent, such as ISSR markers have a maximum value of 0.5 for $f_i = 0.5$ (De Riek et al., 2001). NTSYS software, version 2.00 (Rohlf, 1998), was used to estimate Nei's genetic distance (Nei, 1972). The produced matrix of distances was used to generate a dendrogram by UPGMA method and to perform PCA.

3. Results

3.1. Means and ranges of quantitative variables

Significant differences among the seven collected landraces and fig wild form accessions was observed at $P < 0.01$ in seven quantitative traits (leaf length, leaf area, length of leaf stalk/leaf length, fruit weight, fruit width, fruit length and fruit flesh thickness) and at $P < 0.05$ for leaf width and petiole length (Table 3). Across the

Table 4
Mean for 9 quantitative traits for the 7 Jordanian fig landrace landraces and fig wild forms.

Genotype	Fruit weight	Fruit width	Fruit length	Fruit flesh thickness	Leaf length	Leaf width	Leaf area	Leaf stalk/leaf length	Petiole length
Mwazee	18.40	3.50	4.90	14.70	15.50	14.50	239.50	1.80	57.50
Kortomanee	35.30	4.60	4.60	19.08	25.78	18.40	474.08	1.57	74.50
Zrakee	21.40	3.90	3.25	16.20	17.30	17.35	302.79	0.96	32.00
Ajlonee	9.60	2.90	3.60	11.20	15.30	14.83	230.49	1.59	59.60
Kdaree	21.90	4.20	3.50	17.50	18.80	15.75	305.48	1.73	65.30
Byadee	27.90	4.60	4.00	18.20	20.95	18.87	400.13	1.83	97.30
Hmaree	23.00	3.90	3.90	14.90	16.00	13.84	222.39	2.40	59.10
Wild type	7.50	2.60	2.70	10.90	14.60	14.30	214.60	2.40	63.40
Overall mean	20.63	3.78	4.37	15.34	18.03	16.00	298.69	1.78	63.59

30 fig accessions studied, fruit weight, petiole length and leaf area were with higher variation (CV > 31%), while leaf width and fruit length showed comparatively low values (CV < 19%). The remaining four traits (leaf length, length of leaf stalk/leaf length, fruit weight and fruit flesh thickness) exhibited intermediate CV values; ranging from 14.3 to 28.4% (Table 3).

'Kortomanee', 'Byadee' and 'Kdaree' had longest leaves with 25.78, 20.95, and 18.80 cm, respectively (Table 4), while 'Ajlounee' (15.3 cm) and fig wild forms (14.6 cm) had the shortest leaves. The widest leaves were observed in 'Byadee' (18.87 cm), 'Kortomanee' (18.40 cm) and 'Zrakee' (17.35 cm), whereas the lowest values were obtained in 'Hmaree' (13.84 cm) and fig wild forms (14.3 cm). The maximum leaf area was observed in 'Kortomanee' (474.08 cm^2), followed by 'Byadee' (400.13 cm^2) and 'Zrakee' (302.79 cm^2) landraces, while the minimum leaf area was recorded in 'Hmaree' (222.39 cm^2) and fig wild forms (214.60 cm^2). The highest leaf stalk/leaf length ratio was observed in 'Hmaree' landrace and fig wild forms (the average = 2.4 mm), whereas the lowest value was recorded in 'Zrakee' (0.96 mm). The longest petioles were recorded in 'Byadee' (97.3 mm) and 'Kortomanee' (74.5 mm), while the shortest ones were present in 'Zrakee' landrace (32 mm).

Fruit weight ranged over accessions from 7.5 to 35.3 g/fruit (Table 4). 'Kortomanee' had the heaviest fruits (35.3 g/fruit), followed by 'Byadee', 'Hmaree', and 'Zarakee' with 27.9, 21.90 and 21.40 g, respectively. The lightest fruits were observed in fig wild forms (range = 3.50–10.6 g) and in 'Ajlounee' landrace (9.62 g). The widest fruits were present in 'Kortomanee' and 'Byadee' (4.6 cm) and 'Kdaree' (4.2 cm), while fig wild forms (range = 2.1–3.0 cm) and 'Ajlounee' (3.3 cm) were with narrowest fruits. Long fruits were observed in 'Mwazee' (4.9 cm), 'Kortomanee' (4.60 cm) and 'Byadee' (4.0 cm), while 'Zrakee' (3.25 cm) and wild forms (range = 2.50–3.0 cm) had the shortest fruits. Fruit flesh was thick in 'Kortomanee' (19.08 mm) followed by 'Byadee' (18.20 mm), 'Kdaree' (17.50 mm) and 'Zrakee' (16.2 mm), whereas fig wild type (10.9 mm) and 'Ajlounee' (11.2 mm) had the thinnest flesh.

3.2. Ranges of qualitative traits variables

All qualitative traits were polymorphic showing more than two phenotypes in fig collections, except leaf margin dentation trait which was always with lobes side completely dentated in all accessions. All results obtained from qualitative scoring are presented in Table 5 for leaf related traits and in Table 6 for pomological traits.

The leaf shape was highly polymorphic among fig landraces. 'Mwazee' and 'Kdraee' were with calcarate base and latate lobes, while four landraces (Zrakee, Hmaree, Ajlounee and Byadee) were with cordate base and spatulate lobes, and one (Kortomanee) was with decurrent base and latate lobes. In fig wild forms, 'W3' and 'W6' were with decurrent base and latate lobes, 'W2' and 'W4' were with calcarate base and latate lobes, 'W1' was with truncate base and spatulate lobes and 'W5' was with cordate base and spatulate lobes. All landraces had five lobes per leaf, except 'Kortmanee' with three lobes. High level of polymorphism was observed in fig wild forms in number of lobes per tree; four accessions (W1, W2, W4 and W5) were with five lobes per leaf, while 'W6' and 'W3' had 3 and 7 lobes, respectively. Little lateral lobes were absent in all landraces, except 'Ajlounee' landrace harbored additional lateral lobes. Two fig wild forms (W1 and W4) were with central lobes, one accession (W3) was with lateral lobes and the rest had no lateral lobes. Three phenotypic classes were found in studied accessions. 'Kdaree', 'Kortomanee', 'Ajlounee', 'W2' and 'W4' were dentate, while five genotypes (Byadee, Zrakee and Hmaree, W1 and W3) were undulate and the rest (Mwazee, W5 and W6) were serrate. All studied fig accessions had dark green leaf color, except one fig wild accession 'W3' was green. All landraces had green petioles except 'Kortomanee' with light green color petioles. Conversely, all

Table 5
Scores for qualitative leaf-related traits recorded from 7 fig landraces and 6 fig wild form accessions from Jordan.

Genotype	Leaf shape	Shape of lobes	Shape of leaf base	Number of lobes	Location of little lateral lobes	Leaf margin dentations	Leaf margin	Leaf color	Petiole color
Mwazee	Base calcarate, lobes latate	Latate	Calcarate	Five	Absent	Lobes side completely dented	Serrate	Dark green	Green
Kortomanee	Base decurrent, lobes lyrate	Latate	Decurrent	Three	Absent	Lobes side completely dented	Dentate	Dark green	Light green
Zrakee	Base cordate, lobes spatulate	Spatulate	Cordate	Five	Absent	Lobes side completely dented	Undulate	Dark green	Green
Ajlonee	Base cordate, latate lobes	Latate	Cordate	Five	In lateral lobes	Lobes side completely dented	Dentate	Dark green	Green
Kdaree	Base calcarate, lobes lyrate	Lyrate	Calcarate	Five	Absent	Lobes side completely dented	Dentate	Dark green	Green
Byadee	Base cordate, lobes spatulate	Spatulate	Cordate	Five	Absent	Lobes side completely dented	Undulate	Dark green	Green
Hmaree	Base cordate, lobes spatulate	Spatulate	Cordate	Five	Absent	Lobes side completely dented	Undulate	Dark green	Green
Wild type1	Base truncate, lobes spatulate	Spatualte	Truncate	Five	In central lobes	Lobes side completely dented	Undulate	Dark green	Light green
Wild type2	Base calcarate, lobes latate	Latate	Calcarate	Five	Absent	Lobes side completely dented	Dentate	Dark green	Light green
Wild type3	Base decurrent, lobes latate	Latate	Decurrent	Seven	In lateral lobes	Lobes side completely dented	Undulate	Green	Light green
Wild type4	Base calcarate, lobes linear	Linear	Calcarate	Five	In central lobes	Lobes side completely dented	Dentate	Dark green	Green
Wild type5	Base cordate, lobes spatulate	Latate	Cordate	Five	Absent	Lobes side completely dented	Serrate	Dark green	Light green
Wild type6	Base decurrent lobes latate,	Latate	Deccurent	Three	Absent	Lobes side completely dented	Serrate	Dark green	Light green

Table 6
Scores for qualitative morphopomological traits recorded from 7 fig landraces and 6 fig wild type genotypes from Jordan.

Genotype	Fruit shape	Fruit symmetry	Shape of the fruit stalk	Fruit apex shape	Uniformity of fruit size	Easy of peeling	Fruit skin crack
Mwazee	Oblong	Non symmetric	Long and slender	Acute	Uniform	Difficult	Scares cracks longitudinal
Kortomanee	Globose	Symmetric	Short and thick	Acute	Uniform	Easy	Scares cracks longitudinal
Zrakee	Oblate	Symmetric	Long and slender	Acute	Uniform	Easy	Scares cracks longitudinal
Ajlonee	Oblong	Symmetric	Long and slender	Acute	Uniform	Easy	Scares cracks longitudinal
Kdaree	Oblate	Symmetric	Short and thick	Rounded	Uniform	Easy	Scares cracks longitudinal
Byadee	Oblate	Symmetric	Short and thick	Acute	Uniform	Easy	Scares cracks longitudinal
Hmaree	Globose	Symmetric	Short and thick	Acute	Uniform	Medium	Scares cracks longitudinal
Wild type1	Globose	Symmetric	Long and slender	Rounded	Uniform	Medium	Scares cracks longitudinal
Wild type2	Oblong	Non symmetric	Short and thick	Acute	Uniform	Medium	Minute cracks
Wild type3	Globose	Non symmetric	Short and thick	Acute	Variable	Medium	Minute cracks
Wild type4	Oblong	Non symmetric	Short and thick	Rounded	Uniform	Easy	Scares cracks longitudinal
Wild type5	Oblong	Non symmetric	Short and thick	Rounded	Uniform	Easy	Scares cracks longitudinal
Wild type6	Globose	Symmetric	Short and thick	Flat	Uniform	Easy	Minute cracks

Genotype	Color of scales around the ostiolum	Resistance to ostiole-end crack	Fruit skin ground color	Fruit lenticels quantity	Fruit lenticels color	Pulp flavor	Pulp internal color
Mwazee	Same as skin color	Resistant	Yellow green	Intermediate	Green	Little flavor	Pink
Kortomanee	Same as skin color	Intermediate	Purple	Numerous	Pink	Little flavor	Amber
Zrakee	Different from skin color	Intermediate	Purple	Intermediate	Pink	Aromatic	Amber Oblate
Ajlonee	Different from skin color	Resistant	Yellow green	NO	-	Little flavor	Red Oblate
Kdaree	Same as skin color	Intermediate	Green	Intermediate	White	Little flavor	White Globose
Byadee	Same as skin color	Intermediate	Green	NO	-	Little flavor	Amber Oblate
Hmaree	Different from skin color	Resistant	Green	Numerous	White	Little flavor	Amber Oblate
Wild type1	Same as skin color	Resistant	Purple	Numerous	Pink	Little flavor	Red
Wild type2	Same as skin color	Resistant	Light green	Intermediate	White	Little flavor	Amber Oblate
Wild type3	Same as skin color	Resistant	Light green	Intermediate	White	Little flavor	Amber Oblate
Wild type4	Same as skin color	Resistant	Yellow green	Intermediate	White	Neutral	White Globose
Wild type5	Same as skin color	Resistant	Yellow green	Intermediate	White	Neutral	White Globose
Wild type6	Same as skin color	Resistant	Light green	Numerous	White	Little flavor	Dark red Oblong

fig wild form accessions had light green petioles, except 'W4' with green petioles.

The fruit shape was highly variable among landrace collections. 'Mwazee' and 'Ajlunee' landrace were oblong, while three landraces were oblate (Kdaree, Zrakee and Byadee) and the rest (Kortomane and Hmaree) were globose. Fruits were symmetrical in all landraces except in 'Mwazee' and four fig wild forms (W2, W3, W4 and W5) were not symmetric. Two phenotypic classes for fruit shape were recorded in fig wild forms; 'W2', 'W4' and 'W5' were oblong, whereas the rest were globose. All fig wild type were not symmetric except 'W6' with symmetrical fruits. All landraces had acute fruit apex except 'Kdaree' had round apex. Fruit stalk shape was short and thick in four landraces (Kdaree, Koortomaee, Byadee and Hmaree), whereas the rest were with long selender fruit stalk. All fig wild forms were monomorphic for this trait with short-thick stalk, except 'W1' with long and slender stalk. All landrace accessions had acute fruit apex shape, except 'Kdaree' landrace with round apex. Three phenotypic classes were found in wild fig forms for fruit apex shape, it was acute in 'W2' and 'W3', round in 'W1', 'W4' and 'W5', and flat in 'W6'. All fig collections had uniform fruits size except 'W3' had variable fruit sizes.

The majority of landraces was ease to peel, except 'Mwazee' and 'Hmaree' landraces were with medium and difficult fruit peeling, respectively. Peeling was highly polymorphic among fig wild form; 'W4', 'W5' and 'W6' were easy to peel, while 'W1', 'W2' and 'W3' were with medium fruit peeling. There were no differences in fruit skin cracking in landrace collections; all landraces were with scarce longitudinal cracks. Polymorphism was detected in skin cracking in fig wild form accessions, scarce longitudinal cracks was recorded in 'W1', 'W4' and 'W5', whereas 'W2', 'W3' and 'W6' with minute or scarce cracks. Four landraces and all fig wild forms had the same scale color as the skin (Mwazee, Kdaree, Kortomanee and Byadee), while 'Zrakee', 'Hmaree' and 'Ajlounee' had different scales color from that observed in skin. Three landraces (Mwazee, Hmaree and Ajlouni) and all wild form accessions were resistance to ostiol-end crack while the rest was with intermediate resistant. Two landraces had yellow-green skin colors (Mwazee and Ajlounee), three landraces were green (Kdaree, Byadee and Hmaree), while Kortomanee and Zrakee were with purple skins. Two accessions (W4 and W5) of wild fig forms were yellow green, three light green (W2, W3 and W6), and one (W1) was purple. Lenticels were absent in 'Byadee' and 'Ajlounee', intermediate in three landraces (Mwazee, Kdaree and Zrakee) and numerous in 'Kortomanee' and 'Hmaree'. Two phenotypic classes were found in fig wild forms for lenticels quantity; 'W1' and 'W6' were with numerous lenticels, while the rest were with intermediate lenticels. Fruit lenticels color ranged from green to white-green. 'Hmaree', 'Kdaree' and W2 to W6 were with white lenticels, 'Kortomanee', 'Zrakee' and 'W1' with pink lenticels, and 'Mwazee' landrace was with green lenticels.

Little variation was detected in pulp flavor, one landrace 'Zrakee' was aromatic, while the rest with little flavor. Most fig wild forms were with little flavor except 'W4 and W5' with neutral flavor. Pulp internal color in landraces ranged from white to red. Four landraces were amber (Kortomanee, Byadee, Zrakee and Hmaree), while 'Mwazee', 'Kdraee' and 'Ajlounee' had white, pink and red internal pulps, respectively. Pulp color was a highly polymorphic trait in fig wild forms, 'W2' and 'W3' had amber pulp color, 'W4' and 'W5' had white pulps, while 'W1' and 'W6' had red and dark red pulps, respectively.

3.3. Clustering and principle component analyses for phenotypic data

Euclidean distance coefficients were calculated based on 31 quantitative and qualitative traits (Table 7). The Euclidean distance between accessions ranged from 0.90 to 9.80, with a mean of 6.91. The closest accessions (Euclidean distance = 0.9) were Kd3 with Kd4, while the least similar (Euclidean distance = 9.80) accessions were Kr3 with Aj2. At 10.1 ED, dendogram divided fig accessions into two main clusters (A and B) and two subgroups existed within

Table 7
Euclidean distance based on all morphological data for 24 landrace accessions and 6 fig wild type genotypes; explanation of accession abbreviations is shown in Table 1.

	Mw1	Mw2	Mw3	Kr1	Kr2	Kr3	Kr4	Zr1	Zr2	Aj1	Aj2	Aj3	Aj4	Kd1	Kd2	Kd3	Kd4	By1	By2	By3	By4	Hm1	Hm2	Hm3	W1	W2	W3	W4	W5	W6
Mw1	0																													
Mw2	3.1	0																												
Mw3	1.3	3.1	0																											
Kr1	8.3	9.5	8.7	0																										
Kr2	8.5	9.8	8.9	1.8	0																									
Kr3	8.6	9.9	8.6	1.7	2.1	0																								
Kr4	8.2	9.0	8.6	1.9	2.3	2.3	0																							
Zr1	8.3	8.7	8.3	8.1	8.7	8.3	7.9	0																						
Zr2	9.0	9.1	9.3	8.5	8.9	8.5	7.9	3.3	0																					
Aj1	7.7	8.2	7.6	9.4	9.8	9.4	9.2	7.6	8.3	0																				
Aj2	7.6	7.9	7.5	9.7	1.0	9.8	9.5	7.6	8.3	1.4	0																			
Aj3	8.0	8.2	7.9	1.0	1.1	1.0	9.8	7.7	8.4	1.5	1.8	0																		
Aj4	8.2	8.2	7.9	1.1	1.1	1.1	1.1	8.1	9.4	3.1	2.5	2.6	0																	
Kd1	7.5	8.2	7.4	7.4	8.1	7.7	7.4	6.8	8.2	7.3	7.2	7.4	7.4	0																
Kd2	7.2	8.1	7.3	6.2	6.7	6.4	6.2	6.6	7.7	7.1	7.2	7.5	8.0	2.0	0															
Kd3	7.4	8.4	7.5	6.4	7.0	6.5	6.4	6.7	7.8	7.0	7.3	7.6	8.0	2.4	1.2	0														
Kd4	7.3	8.3	7.4	6.1	6.6	6.1	6.0	6.8	7.6	7.0	7.3	7.5	8.1	2.8	1.2	0.9	0													
By1	8.3	9.2	8.4	7.0	7.3	7.1	7.0	5.7	6.6	7.2	7.5	7.7	8.5	6.4	5.5	5.5	5.5	0												
By2	8.9	1.0	9.1	6.8	7.1	6.8	7.2	6.7	7.5	7.8	8.4	8.6	9.6	7.3	6.2	6.0	5.9	2.3	0											
By3	9.0	1.0	9.3	6.7	7.0	6.6	6.9	6.8	7.1	7.8	8.4	8.6	9.8	7.6	6.3	6.3	6.0	2.8	1.5	0										
By4	8.1	8.8	8.3	7.2	7.5	7.6	7.1	5.6	6.6	7.3	7.4	7.9	8.2	6.1	5.5	5.8	5.7	1.5	3.4	3.9	0									
Hm1	6.8	7.5	6.8	7.9	8.2	8.1	7.8	5.3	7.0	6.7	6.6	7.1	7.0	5.6	5.6	5.8	5.9	5.5	6.5	6.6	5.1	0								
Hm2	6.9	7.3	6.8	8.4	8.7	8.7	8.2	5.3	7.0	6.8	6.7	7.0	6.7	5.6	5.8	6.0	6.1	5.7	6.9	7.2	5.2	1.5	0							
Hm3	7.3	7.9	7.3	7.6	8.2	8.0	7.7	5.8	7.4	7.2	7.5	7.6	7.8	6.3	6.0	5.9	6.0	5.2	6.0	6.4	5.2	3.0	2.8	0						
W1	8.0	8.7	7.6	8.5	9.1	8.3	8.4	7.0	8.5	7.2	7.6	7.3	7.6	7.2	7.3	7.0	7.2	7.7	8.2	8.4	8.0	6.4	6.4	6.4	0					
W2	7.8	8.7	7.2	8.9	9.4	8.7	9.0	9.2	1.1	8.1	8.5	8.2	8.3	7.1	7.3	7.0	7.2	8.5	8.8	9.3	8.7	7.5	7.4	7.2	7.0	0				
W3	9.3	9.9	9.2	1.1	1.1	1.1	1.1	1.1	1.2	9.7	9.8	9.7	1.0	9.7	9.7	9.6	9.7	1.0	1.1	1.1	1.1	10	10	1.0	9.5	8.2	0			
W4	8.5	8.8	8.0	1.1	1.1	1.0	1.0	9.7	1.1	7.8	7.9	7.7	7.4	6.4	7.1	6.8	7.0	8.8	9.5	9.8	8.8	7.4	7.2	7.7	7.7	6.4	1.0	0		
W5	8.9	8.8	8.7	1.1	1.1	1.1	1.0	1.0	1.1	8.3	8.1	7.5	7.0	7.8	7.7	7.7	7.9	9.5	1.1	1.1	9.2	7.9	7.6	8.6	7.8	7.3	1.0	5.1	0	
W6	9.5	9.6	9.2	9.7	1.0	9.8	9.5	9.6	1.1	8.9	8.7	8.9	8.5	7.0	7.7	7.7	7.9	9.9	1.1	1.1	9.6	8.5	8.3	9.2	7.2	7.4	1.0	8.3	7.5	0

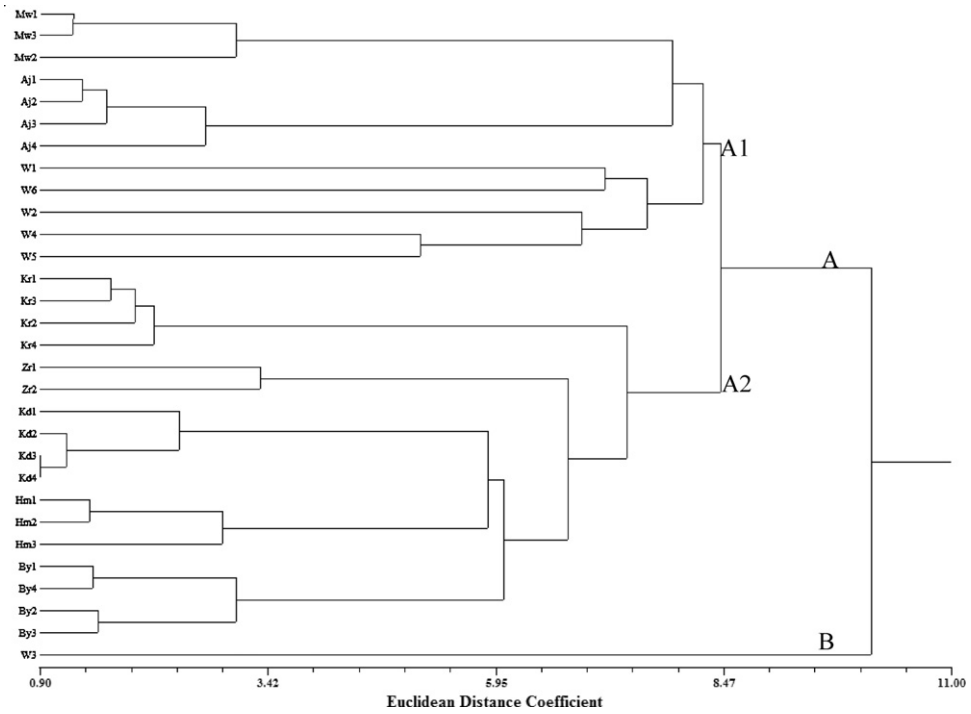

Fig. 1. UPGMA dendogram of 24 landrace accessions and 6 fig wild type genotypes based on 31 quantitative and qualitative traits; explanation of accession abbreviations is shown in Table 1.

cluster (A), A1 and A2 (Fig. 1). The first main cluster (A) included all fig collections except 'W3' from fig wild form, which was placed in an isolated cluster (cluster B). The first subgroup (A1) within cluster A contained all 'Mwazee' and 'Ajlouni' accessions as well as the other five fig wild form accessions (W1, W2, W4, W5 and W6), whereas the second subgroup (A2) included 'Kortomanee', 'Zrakee', 'Kdaree', 'Hmaree' and 'Byadee' accessions. A1 subgroup contained two sub-subgroup, the first included 'Mwazee' and 'Ajlounee' accessions, while the second sub-subgroup comprised from the five fig wild form accessions (W1, W2, W4, W5 and W6). Two sub-subgroups were also existed within A2 subgroup; one contained 'Kortomanee' accessions and the other contained 'Zrakee', 'Hmaree', 'Kdaree' and 'Byadee' accessions. 'W3' was unique with some of its qualitative traits, it had seven lobes per leaf and green leaf color with no uniformity in fruits size, while the other main group (A) showed 3–5 lobes per leaf, dark green leaf color and uniform fruit size. Subgroup A2 on average characterized in comparison with subgroup A2 by large-sized fruits (fruits are heavier, longer and wider), large-sized leaves (wider and longer leaves with elongated petioles). Moreover, A1 subgroup had almost oblong fruit shape and high leaf stalk length/leaf length in comparison with A2 subgroup. In general, clustering based on all morphological data showed that fig wild type accessions were more related to 'Mwazee' and 'Ajlounee'.

Principle component analysis was performed for 30 Jordanian fig accessions. The first five functions account for 74.18% of the total variation (Table 8). The first function accounted for 27.25%, which is strongly influenced by fruit weight and width, fruit flesh thickness, fruit shape, fruit skin ground color, leaf length and width, leaf area, number of lobes, location of little lateral lobes and resistance of ostiole-end crack. The second function accounted for 16.55% of total variation and is mainly explained by shape of leaf base, petiole color, color of scales around ostilum and fruit lenticels quantity. The third function accounted for 12.13% of the total variation, which is mainly explained by uniformity of fruit size, fruit apex shape, shape of fruit stalk, easiness of peeling and fruit length.

3.4. Clustering and principle component analyses for ISSR data

A total of 22 ISSR bands were amplified using the five selected ISSR primers, of which 20 (90%) were polymorphic (Table 1). The number of total amplified bands varied from three in IMA5 to six in IMA834, with fragment size ranged from 250 to 1366 bp. The five ISSR primers generated 660 data points, among which 516 data entries were for present bands (1) and 144 for absent bands (0). Bands with the same molecular weight and mobility were treated as identical fragments. Three to five polymorphic bands were generated with a mean of 4.0 bands per primer (Table 2). The PIC values of ISSR markers varied from 0.193 in IMA5 to 0.300 in IMA12 (Table 2).

Nei's genetic distance (D) identifies the distance between two genotypes on scale 0 to 1. The obtained matrix exhibits genetic distances ranging from 0.00 to 0.830, with a mean of 0.637 (Table 9). These distances express high genetic diversity among local fig germplasm at the DNA level. Genetic distance of zero was observed between 'Mwazi' landrace accessions, Zr1 and Zr2, By1 and By2, Aj2 and Aj4 and 'W2', 'W3' and 'W4'. However, the most dissimilar accessions were Aj3 with Kr4 and three fig wild form accessions (W2, W3 and W4).

The dendrogram separated the fig accessions into two main groups, A and B (Fig. 2), one comprised from Aj3 and W1 (B group), and the other group (A) contained all other accessions. Two sub-groups were identified within the second cluster. The first subgroup (A1) contained all accessions of 'Mwazee', 'Kortomanee', 'Zrakee', 'Kdaree' and 'Hmaree' landraces, two accessions from 'Byadee' (By1 and By2), three accessions from 'Ajlouni' landrace (Aj1, Aj2 and Aj4) and five fig wild form accessions (W2, W3, W4, W5 and W6). The second subgroup (A2) contained two accessions from 'Byadee' landrace (By1 and By2), which placed in isolated group within the second cluster. The first subgroup contained three sub-subgroups: the first sub-subgroup contained all 'Mwazee', 'Zrakee' and 'Kortomanee' accessions, the second sub-subgroup contained 'Kdraee' and 'Hmaree' accessions in addition to the two other 'Byadee' accessions (By1 and By2), and the third

Table 8
Correlation coefficients between the morphological characters and the first five principle components for 24 landrace accessions and 6 fig wild forms from Jordan.

Traits	Function				
	1	2	3	4	5
Leaf length	0.86	0.10	0.10	0.29	0.04
Leaf width	0.73	0.14	0.14	0.33	0.35
Leaf area	0.86	0.04	0.12	0.32	0.15
Length of leaf stalk/length of leaf	0.29	0.24	0.35	0.31	0.40
Petiole length	0.45	0.20	0.42	0.17	0.34
Fruit weight	0.92	0.01	0.24	0.07	0.02
Fruit width	0.89	0.12	0.14	0.11	0.04
Fruit length	0.43	0.04	0.71	0.07	0.13
Fruit flesh thickness	0.88	0.10	0.03	0.12	0.20
Leaf shape	0.01	0.60	0.14	0.64	0.20
Shape of lobes	0.22	0.59	0.06	0.32	0.40
Shape of leaf base	0.36	0.76	0.17	0.24	0.00
Number of lobes	0.67	0.52	0.9	0.27	0.26
Location of little lateral lobes	0.57	0.24	0.60	0.70	0.08
Leaf color	0.17	0.26	0.09	0.35	0.69
Petiole color	0.18	0.70	0.14	0.35	0.07
Fruit shape	0.67	0.29	0.34	0.19	0.13
Fruit symmetry	0.51	0.53	0.28	0.23	0.35
Shape of fruit stalk	0.44	0.33	0.65	0.17	0.28
Fruit apex shape	0.24	0.35	0.65	0.16	0.31
Easy of peeling	0.34	0.30	0.65	0.43	0.29
Fruit skin crack	0.23	0.45	0.19	0.25	0.44
Color of scales around the ostiolum	0.28	0.69	0.19	0.09	0.19
Resistance to ostiole- end crack	0.84	0.20	0.01	0.13	0.06
Fruit skin ground color	0.83	0.02	0.06	0.02	0.08
Fruit lenticels quantity	0.18	0.66	0.03	0.39	0.15
Fruit lenticels color	0.14	0.57	0.60	0.36	0.20
Pulp flavor	0.35	0.39	0.41	0.06	0.05
Pulp internal color	0.42	0.15	0.35	0.46	0.20
Uniformity of fruit size	0.31	0.39	0.71	0.12	0.33

sub-subgroup comprised from five fig wild form accessions (W2, W3, W4, W5 and W6). Principle component analysis was performed for 30 Jordanian fig accessions. The first three functions account for 53.37% of the total variation. The first three functions account for 20.43, 20.01 and 12.93% of the total genetic variation.

4. Discussion

4.1. Morphological characterization

For the purpose of this study, 9 quantitative and 22 qualitative traits were selected to characterize Jordanian fig germplasm and to

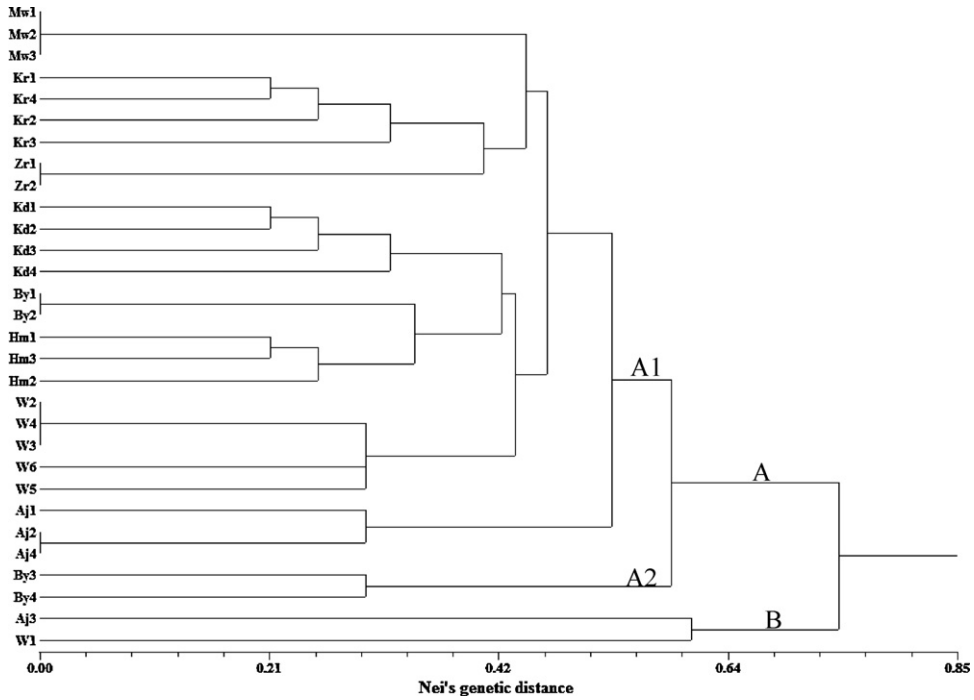

Fig. 2. UPGMA dendogram of 24 landrace accessions and 6 fig wild type genotypes based on ISSR banding patterns; explanation of accession abbreviations is shown in Table 1.

Table 9
Nei genetic distance matrix among 24 landrace accessions and 6 fig wild forms based on ISSR data; explanation of accession abbreviations is shown in Table 1.

	Mw1	Mw2	Mw3	Kr1	Kr2	Kr3	Kr4	Zr1	Zr2	Aj1	Aj2	Aj3	Aj4	Kd1	Kd2	Kd3	Kd4	By1	By2	By3	By4	Hm1	Hm2	Hm3	W1	W2	W3	W4	W5	W6
Mw1	0																													
Mw2	0	0																												
Mw3	0	0	0																											
Kr1	0.43	0.43	0.43	0																										
Kr2	0.43	0.43	0.43	0.3	0																									
Kr3	0.52	0.52	0.52	0.3	0.3	0																								
Kr4	0.37	0.37	0.37	0.21	0.21	0.37	0																							
Zr1	0.48	0.48	0.48	0.37	0.48	0.37	0.43	0																						
Zr2	0.48	0.48	0.48	0.37	0.48	0.37	0.43	0	0																					
Aj1	0.52	0.52	0.52	0.52	0.52	0.6	0.48	0.56	0.56	0																				
Aj2	0.52	0.52	0.52	0.52	0.6	0.6	0.56	0.56	0.56	0.3	0																			
Aj3	0.78	0.78	0.78	0.78	0.78	0.74	0.83	0.77	0.77	0.67	0.6	0																		
Aj4	0.52	0.52	0.52	0.52	0.6	0.6	0.56	0.56	0.56	0.3	0	0.6	0																	
Kd1	0.43	0.43	0.43	0.43	0.43	0.52	0.37	0.48	0.48	0.43	0.52	0.8	0.52	0																
Kd2	0.48	0.48	0.48	0.48	0.48	0.56	0.43	0.43	0.43	0.37	0.48	0.78	0.48	0.21	0															
Kd3	0.52	0.52	0.52	0.43	0.43	0.52	0.37	0.48	0.48	0.3	0.43	0.74	0.43	0.3	0.21	0														
Kd4	0.52	0.52	0.52	0.43	0.43	0.43	0.37	0.48	0.48	0.43	0.52	0.74	0.52	0.3	0.3	0.3	0													
By1	0.48	0.48	0.48	0.48	0.37	0.48	0.43	0.52	0.52	0.56	0.64	0.77	0.64	0.37	0.43	0.48	0.48	0												
By2	0.48	0.48	0.43	0.48	0.37	0.48	0.43	0.52	0.52	0.56	0.64	0.77	0.64	0.37	0.43	0.48	0.48	0	0											
By3	0.6	0.6	0.6	0.67	0.6	0.6	0.64	0.64	0.64	0.74	0.74	0.74	0.74	0.6	0.64	0.67	0.64	0.48	0.48	0										
By4	0.52	0.52	0.52	0.6	0.52	0.6	0.56	0.64	0.64	0.67	0.67	0.8	0.67	0.52	0.56	0.6	0.6	0.37	0.37	0.3	0									
Hm1	0.48	0.48	0.48	0.37	0.37	0.48	0.3	0.52	0.52	0.48	0.56	0.77	0.56	0.37	0.43	0.37	0.37	0.3	0.3	o.56	0.48	0								
Hm2	0.43	0.48	0.43	0.43	0.43	0.52	0.37	0.56	0.56	0.43	0.52	0.74	0.52	0.43	0.48	0.43	0.43	0.37	0.37	0.6	0.52	0.21	0							
Hm3	0.52	0.52	0.52	0.43	0.43	0.52	0.37	0.56	0.56	0.52	0.6	0.8	0.6	0.43	0.48	0.43	0.43	0.37	0.37	0.52	0.43	0.21	0.3	0						
W1	0.74	0.74	0.74	0.74	0.67	0.67	0.71	0.77	0.77	0.8	0.8	0.6	0.8	0.74	0.77	0.74	0.67	0.64	0.64	0.67	0.67	0.64	0.67	0.67	0					
W2	0.48	0.48	0.48	0.48	0.48	0.56	0.43	0.52	0.52	0.48	0.56	0.83	0.56	0.37	0.43	0.48	0.48	0.43	0.43	0.64	0.56	0.43	0.48	0.48	0.71	0				
W3	0.48	0.48	0.48	0.48	0.48	0.56	0.43	0.52	0.52	0.48	0.56	0.83	0.56	0.37	0.43	0.48	0.48	0.43	0.43	0.64	0.56	0.43	0.48	0.48	0.71	0.	0			
W4	0.48	0.48	0.48	0.48	0.48	0.56	0.43	0.52	0.52	0.48	0.56	0.83	0.56	0.37	0.43	0.48	0.48	0.43	0.43	0.64	0.56	0.43	0.48	0.48	0.71	0	0	0		
W5	0.37	0.37	0.37	0.48	0.48	0.56	0.43	0.52	0.52	0.48	0.48	0.77	0.48	0.37	0.43	0.48	0.48	0.43	0.43	0.56	0.48	0.43	0.48	0.48	0.71	0.3	0.3	0.3	0	
W6	0.48	0.48	0.48	0.48	0.48	0.48	0.43	0.43	0.43	0.48	0.56	0.77	0.56	0.37	0.43	0.48	0.37	0.43	0.43	0.56	0.56	0.43	0.48	0.48	0.71	0.3	0.3	0.3	0.3	0

investigate the efficacy of morphological traits in genotypes identification. According to Condit (1955) and Papadopoulou et al. (2002), these traits are well defined traits for identifying fig genotypes. Most of these traits are with economic interest especially those related to fruit quality and pest resistance, and consequently serve as target traits for plant breeders and fig growers. Our findings support the view that morphopomological and leaf characteristics are reliable in estimating genetic relationships among large and diverse groups of fig genotypes and can be used efficiently for discrimination. Similarly, many studies (Condit, 1955; Mars et al., 1998; Oukabli et al., 2002; Papadopoulou et al., 2002; Mars, 2003; Aljane and Ferchichi, 2005) revealed that morphological traits are very helpful in identification and evaluation of fig germplasm.

This study showed that Jordanian fig germplasm is a rich source of variation for traits of economic interest. This conclusion came from the high significant differences between Jordanian fig collections for quantitative traits combined with high CV values and the presence of two or more phenotypic classes per trait for qualitative traits. Landrace fig materials used in the current study were collected from their sampling sites and then planted on the same orchard at Al-Mshaqar agricultural station. Therefore, the present phenotypic values do not include differences due to environmental factors. Similarly, high phenotypic variability were reported in international fig collections from Tunisia (Mars et al., 1998; Chatti et al., 2003), Turkey (Caliskan and Polat, 2008), Morroco (Oukabli et al., 2002), Spain (Sánches et al., 2002), Iran (Mahdavian et al., 2006) and Lebanon (Chalack et al., 2005); these studies indicated that high diversity in morphopomological and leaf-related traits could be used as an efficient marker system to discriminate between fig genotypes.

Fruit weight is one of the most important components for determining the size of fruits. In this study fruit weight values in the seven studied landraces ranged from 9.6 to 35.3 g; 'Kortomanee', 'Byadee' and 'Zrakee' landraces had largest fruit weights with 35.3, 27.9 and 21.4 g, respectively. In another study conducted by Ateyyeh and Sadder (2006) on six landraces (Ajlounee, Byadee, Kortomanee and Kdaree, Mwazee and Zrakee), the fruit weights ranged from 11.1 to 28.1 g, the highest values were recorded for 'Zrakee' and 'Mwazee' with 28.1 and 23.1 g, respectively. High variability was recorded in fruit dimensions (width and length). Fruit index or fruit shape (width/length) is of great importance in packing and transportation (Condit, 1941; Caliskan and Polat, 2008). The fruit shape of Jordanian fig collections ranged between oblate and oblong. 'Kortomanee' and 'Hmaree' landraces as well as one fig wild accession 'W1' were with globose shape, which is the most suitable fruit shape for packing and transportation (Condit, 1941; Caliskan and Polat, 2008).

Considerable variability was also recorded in Jordanian fig germplasm for fruits lenticels color and quantity as well as fruit apex shape and fruit skin cracks, thus indicating again a wide genetic variability. Skin fruit color and pulp flavor and internal color are critical for fresh fig consumption and consumers preferences (Mars et al., 1998; Caliskan and Polat, 2008). In this study, high variations were observed in skin fruit and internal pulp colors. Overall fig accessions, fruit skin color ranged from yellow-green to purple, whereas pulp internal color ranged from white to red in landraces and from white to dark red in fig wild form. One landrace 'Zrakee' was aromatic, while the rest with little or neutral flavor. Fruit uniformity and fruit symmetry are also very important traits for packaging and transportation (Condit, 1941); most fig collections under study were uniform and symmetrical. Ease of peeling and fruit skin color are also two important traits relate to preferences for fig fresh consumption and customer preferences (Can, 1993; Ilgin, 1995). Fruits of five landraces (Kortomanee, Zrakee, Ajlounee, Kdraee and Byadee) and three wild fig form accessions were easy to peel. 'Mwazee', 'Hmaree' and 'Ajlounee' landraces as well as fig wild type forms were resistant to ostiolum end crack. Cracking around ostiolum is an undesirable characteristic, as pests and pathogens enter the fruit via these cracks (Can, 1993).

Besides the variability in morphopomological traits in Jordanian fig germplasm, high variability were also recorded in leaf-related traits such as leaf size (length, width and area), leaf shape, lobes shape, number of lobes per leaf. Leaf traits are very important for landrace evaluation and in taxonomic grouping studies (Papadopoulou et al., 2002).

4.2. Genetic polymorphism and ISSR patterns

The proportion of polymorphic loci out of 22 ISSR loci scored was 90%, with an average of 4.4 bands per primer. The percentage of polymorphic bands ranged from 75% in IMA12 to 100% in IMA5 and UBC841. Previous studies on *F. carica* L. (Khadari et al., 2003c; Amel et al., 2004; Ikegami et al., 2009) reported much higher numbers of amplified fragments using similar primers, which might be due to difference in genetic materials. Although limited number of alleles per ISSR primer was detected, 22 different banding patterns were recorded. The PIC values for the five ISSR primers were ranged from 0.19 to 0.30, indicating their intermediate to high discriminating power. ISSR produced a unique fingerprint for most fig landraces/fig wild forms examined in this study and proved to be suitable for the detection of the genetic variability between accessions (i.e. clones) of the same landrace. Similarly, Papadopoulou et al. (2002) reported high level of polymorphism in a collection of 64 *F. carica* L. accessions using RAPD with enough discriminating power to differentiate clones from the same landrace and to give unique fingerprints. According to Khadari et al. (2003c) and Ikegami et al. (2009), ISSR is very powerful and attractive approach for genetic diversity studies which could be used as efficient marker system for differentiation among fig landraces.

4.3. Clustering and principle component analyses

The mean value of Nei's genetic distance was 0.637, ranging from 0.00 to 0.830. In earlier RAPD study, Sadder and Ateyyeh (2006) observed genetic diversity values ranging from 0.10 to 0.97 among 20 different local Jordanian fig genotypes. The five selected ISSR markers revealed wide range of genetic diversity similar to those observed by Sadder and Ateyyeh (2006). The high degree of diversification among landrace and wild fig forms confirmed what was observed at the phenotypic level. However, ISSR markers provided an unbiased estimation of the genetic diversity in absence of the external environmental conditions. The comparison between the two marker systems revealed non-significant correlation coefficients ($r = -0.08$, $p < 0.07$), which could be explained by absence of linkage between markers and loci that control the studied morphological characters. Contrastingly, a study conducted by Papadopoulou et al. (2002) revealed that genetic distances obtained from RAPD were primarily related to geographic origin and to specific phenotypic traits.

The clustering pattern obtained by morphological and ISSR markers showed almost mutually independent results. However, some common groupings were observed in ISSR and morphological traits based on UPGMA clustering. 'Kdaree', 'Hmaree' and some 'Byadee' accessions that were closely clustered in ISSR based dendogram, were also clustered in the morphology based dendogram. Clones derived from 'Kortomanee' and 'Zrakee' were also closely clustered in both analyses. Fig wild form accessions had also somewhat close clustering in ISSR and morphological traits based clustering. Such sort pattern confirming that the names given by farmers to these accessions are consistent. Conversely, genotypes such as 'Mwazee' and 'Ajlouni' clones that were closely clustered in ISSR dendogram, did not do so in morphology-based dendogram,

Table 10
Banding patterns resulting from five ISSR primers for 24 landrace accessions and 6 fig wild forms; explanation of accession abbreviations is shown in Table 1.

Marker	Mw1	Mw2	Mw3	Kr1	Kr2	Kr3	Kr4	Zr1	Zr2	Aj1	Aj2	Aj3	Aj4	Kd1	Kd2	Kd3	Kd4	By1	By2	By3	By4	Hm1	Hm2	Hm3	W1	W2	W3	W4	W5	W6
IMA5-373	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		+	+	+	+	+
IMA5-950	+	+	+	+	+	+	+	+	+	+	+		+	+	+	+	+	+	+	+	+	+	+	+		+	+	+	+	+
IMA5-1238	+	+	+	+	+		+			+	+		+	+	+	+		+	+		+	+	+		+	+	+	+	
UBC841-478				+		+		+	+		+	+	+	+																
UBC841-700	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+						+					+
UBC841-922																														
UBC841-1144	+	+	+	+	+	+	+	+	+	+	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
UBC841-1366	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		+	+	+	+		+	+	+	+	+
IMA12-652	+	+	+	+	+	+	+	+	+	+			+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
IMA12-868	+	+	+	+	+	+	+	+	+																					
IMA12-1190																		+	+	+	+	+	+	+	+	+	+	+	+	+
IMA12-1300	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
UBC818-250	+	+	+	+	+	+	+	+	+	+	+		+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
UBC818-500	+	+	+	+	+	+	+	+	+	+	+		+	+	+	+	+									+	+	+	+	+
UBC818-666	+	+	+	+	+		+	+	+	+	+		+	+	+	+	+					+	+	+		+	+	+	+	+
UBC818-833	+	+	+	+	+	+	+	+	+	+	+		+	+	+	+		+	+	+	+	+	+	+		+	+	+	+	+
IMA834-300	+	+	+	+	+	+	+	+	+	+	+		+	+	+	+		+	+	+	+	+				+	+	+	+	+
IMA834-486	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		+	+	+	+	+
IMA834-671	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+		+	+	+	+	+	+	+
IMA834-764										+	+	+	+	+	+	+	+	+	+	+	+					+	+	+	+	+
IMA834-857										+	+	+	+	+	+	+	+													
IMA834-1230	+	+	+	+	+	+	+	+	+	+	+		+	+	+	+	+	+	+	+	+	+	+	+		+	+	+	+	+

and this was the case for Ajlouni landrace accession 'Aj3' and the two fig wild forms 'W1' and 'W3'. Similarly, Papadopoulou et al. (2002) found similar disparity between morphological and DNA markers clustering in fig.

Clustering based on ISSR data produced one large cluster and one small cluster (cluster B) containing one clone from Ajlounee landrace 'Aj3' and one wild type genotype 'W1'. Similarly, clustering based on morphological data placed one fig wild accession 'W3' in one isolated main cluster. These results may indicate the unique genetic background of these accessions (Aj3, W1 and W3). Most fig wild forms were unlikely clustered within common fig landraces, indicating the high genetic similarity between common and fig wild form germplasm. Similarly, Papadopoulou et al. (2002) and Sadder and Ateyyeh (2006) obtained relatively high genetic similarity values between fig wild form and common fig germplasm using RAPD markers.

Multivariate analysis of traits showed that the first five principle components accounted for 76% of total genetic variation among genotypes. The first three principle components mainly explained by morpho-pomological traits. Many authors; found a large number of morphopomological traits in a set of Tunisian (Aljane and Ferchichi, 2005), Moroccans (Oukabli et al., 2002), Grecian (Papadopoulou et al., 2002) and Turkish (Caliskan and Polat, 2008, 2012) landraces, which could provide enough phenotypic variation that efficient for fig genetic diversity evaluation. The first three functions account for more than 50% of the total variation indicating again the high level of variability could be explained by ISSR markers.

4.4. Implications for gene bank conservations

Screening with the five ISSR primers revealed the presence of the same ISSR allelic profile for accessions from the same landrace. So among 24 common fig accessions and 6 fig wild forms, 11 and 3 trees could be considered as duplicate of the plant materials (Table 10). So ISSR, can provide the basic information necessary to help gene bank to conserve materials from different genetic background rather than duplicates from same clone. 'Ajlounee' and 'Byadee' accessions were distributed in different clusters possibly due to intra-cultivar variability or misnaming. Fig landraces are very common in Jordan and their domination is very complicated because of morphological similarity. Homonymy is a problem in Jordan; the main problem comes from denominating landraces based on common morphopomological traits. So, different landraces could have the same landrace name because they share some morphopomological traits. Discriminating of homonymous cases in fig was reported by Khadari et al. (1995) and Papadopoulou et al. (2002) using RAPD marker.

5. Conclusion

Broad phenotypic diversity was existed among common and fig wild forms from Jordan. High level of variability obtained by Jordanian fig germplasm can be exploited in breeding programs for *F. carica* L. improvement. Many traits recorded in this study are with high economic importance and consequently usually serve as target traits for selection by fig growers and breeders. Information on current levels of genetic diversity of germplasm at gene bank is essential for devising strategies for fig landraces and wild forms conservation. ISSR screening revealed the presence of the same ISSR allelic profile for accessions collected from the same landrace or fig wild forms, indicating that ISSR can provide the basic information necessary to help gene banks to conserve materials from different genetic background rather than duplicates from same clone.

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