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Evaluation of genetic diversity of figs (*Ficus carica* L.) in Sulaymaniyah governorate using morphological, pomological and ISSR molecular marker

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ABSTRACT

The fig (*Ficus carica* L.) is a fruit tree that is important in the Mediterranean region, it is widely distributed in Sulaymaniyah province of the Iraqi Kurdistan region. Due to a lack of information available about the genetic diversity of this plant in Iraq. Thus, in the current study, 12 morphological traits and 15 ISSR markers were used for genetic diversity analysis of 66 fig accessions. Analysis of variance recorded highly significant differences concerning plant morphological and pomological traits in addition to genetic diversity. The highest values for shoot length (79.959 cm), internode diameter (15.563 mm), leaf length (28.183 cm), leaf width (28.480 cm), leaf petiole length (13.397 cm) and leaf petiole diameter (18.360 mm) recorded in AC17, AC41, AC20, AC20, AC17 and AC24, respectively. However, the lowest values (11.120 cm, 4.340 mm, 10.910 cm, 9.813 cm, 3.987 cm and 2.323 mm) for the mentioned traits were recorded in AC14, AC12, AC12, AC12, AC37 and AC52, respectively. The highest values for fruit weight (63.447 g), fruit length (46.960 mm), fruit thickness (60.420 mm), fruit stalk length (29.887 mm), fruit stalk diameter (10.433 mm) and ostiole diameter (8.717 mm) were given by AC03, AC22, AC54, AC58, AC58 and AC14, successively. Whereas the lowest values (4.483 g, 14.770 mm, 18.497 mm, 2.373 mm, 2.533 mm and 2.557 mm) were observed in AC37, AC37, AC12, AC08, AC25 and AC30, successively. The first two principal components analysis (PCA) described 49.15% of the total quality variance. ISSR marker produced 197 polymorphic bands. The genetic diversities ranged as (0.883 to 0.980) and polymorphism information content (PIC) ranged as (0.878 to 0.979), with 100% polymorphism levels. The fig accessions classified into 10 clusters by dendrogram created by ward method. The results indicated that natural fig populations in this region provide a rich genetic resource for fig germplasms, and significant genetic variation across accessions originating from different populations, as well as the marker was informative for genetic variability detection in the collections. The findings of this study could be used in breeding processes.

تقييم التنوع الوراثي للتين (*Ficus carica* L.) في محافظة السليمانية عن طريق الصفات المظهرية والثمرية و دلائل الجزيئية، تكرار التسلسل البسيط

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الخلاصة

التين Fig (*Ficus carica* L.) من الفاكهة المهمة في منطقة البحر الأبيض المتوسط ، وبالتالي أصبح التحسين الوراثي مجالاً مهماً للبحث وتحسين المحاصيل المفضلة ، واخذ المعلومات عن هذا النوع ، لا سيما تنوعه الوراثي المرتبط بالصفات المورفولوجية لأصناف التين المزروعة والأنواع البرية المنتشرة في محافظة السليمانية. جمعت عينات من 66 نوع من التين الموجودة، واستخدمت 15 بادئة لواسمات ISSR لوصف التباين الجيني ، مع 12 صفات المظهري. سجل تحليل التباين اختلافات ذات دلالة إحصائية فيما يتعلق بالصفات المورفولوجية للنبات، الصفات االثمرية والتنوع الوراثي. تم تسجيل أعلى القيم لطول النموات السنوية (79.959 سم) ، قطر النموات السنوية (15.563 ملم) ، طول الورقة (28.183 سم) ، عرض الورقة (28.480 سم) ، طول سويقة الورقة (13.397 سم) وقطر سويقات الورقة (18.360 ملم) في AC17 ، AC41 ، AC20 ، AC20 ، AC17و AC24 على التوالي. ومع ذلك ، تم تسجيل أدنى القيم (11.120 سم ، 4.340 ملم ، 10.910 سم ، 9.813 سم ، 3.987 سم و 2.373 ملم) للصفات المذكورة في AC14 ، AC12 ، AC12 ، AC12 ، AC37 و AC52 على التوالي. أعطيت أعلى القيم لوزن الثمرة (63.447غم) ، طول الثمرة (46.960 ملم)، سمك الثمرة (60.460 ملم)، طول حامل الثمرة (29.887 ملم)، قطر حامل الثمرة (10.433 ملم) وقطر نقرة الثمرة (8.717 ملم) في AC03 ، AC22 ، AC54 ، AC58 ، AC58 و AC14 على التوالي. بينما لوحظت أدنى القيم (4.483 غم ، 14.770 ملم ، 18.497 ملم ، 2.373 ملم ، 2.533 ملم ، 2.557 ملم) في كل من AC37 ، AC37 ، AC12 ، AC08 ، AC25 و AC30 على التوالي. وصف أول تحليل مكونين رئيسيين (PCA) 49.15 ٪ من إجمالي تباين الجودة. أنتجت علامة ISSR 197 نطاقا متعدد الأشكال. تراوحت التنوعات الجينية بين (0.883 إلى 0.980) وتنوع محتوى معلومات تعدد الأشكال (PIC) من (0.878 إلى 0.979) ، مع مستويات تعدد أشكال 100٪. بالإضافة إلى ذلك ، تم تقسيم مدخلات التين إلى 10 عناقيد حسب أشارات النتائج إلى أن مجموعات التين الطبيعية في هذه المنطقة توفرت موردًا وراثيًا غنيًا للبلازما الجرثومية للتين ، كما أن التباين الجيني الكبير عبر المُدخَلات التي نشأت من مجموعات سكانية مختلفة ، بالإضافة إلى أن الواسم كان مفيدًا لاكتشاف التباين الجيني في المجموعات. . يمكن أن تدعم نتائج هذه الدراسة في الحفاظ على الأصول الوراثية للتين وزيادة استخدامها.

الكلمات المفتاحية: التنوع الجيني، الصفات المظهرية، التركيب، العنقود.

INTRODUCTION

Common fig (*Ficus carica* L.), a traditional fruit crop, is a subtropical deciduous fruit in the Moraceae family, suitable for Mediterranean climates and high temperatures (Chithiraichelvan *et al*., 2017). Ficus is the largest angiosperm genera with over 800 plant species. Two sexual forms of the gynodioecious fig plants are present in nature, the pollinator or male tree (caprifig) and the female tree (domesticated fig), which produces the edible fruit (Ikegami *et al.*, 2013; Ali, 2019).

Classical cultivar identification relies on morphological features like fruit size, flesh color, skin color and other vegetative traits. Discrimination of the morphologically comparative cultivars at the molecular level is significant to evaluate fig tree biodiversity and genetic resources (Caliskan *et*

al., 2018; Ali, 2019). Morphological parameters and molecular markers reveal phenotypic and genetic inconsistencies in edible fig germplasm (Giraldo *et al.,* 2010). Genetic diversity may be a fundamental component of biodiversity and its preservation is basic for long term survival of any species in changing environment (Kumar and Agrawal, 2019).

DNA-based markers have been shown to be useful in determining genetic diversity and classifying plants (Zhou *et al.,* 2013; Abdelsalam *et al.,* 2019), while they are influenced by environmental conditions. Besides, the morphological and agronomic features are useful instruments for the survival of the diversity of plant species. To estimate the genetic polymorphism, and study the genetic diversity of fig germplasm, a wide variation of molecular markers were used (Simsek *et al.,* 2017; Khadivi *et al.,* 2018). Markers such as Inter Simple Sequence Repeat (ISSR) and Start Codon Targeted polymorphism (SCoT) were utilized effectively for genetic diversity assessment of plants (Abd El-Aziz *et al.,* 2019; Rasul *et al.,* 2022). The Inter Simple Sequence Repeat (ISSR) marker is a basic, easy, and fast test to perform. It offers several advantages over other overwhelming markers, and this way has been widely used for plant genomic analysis (Al-Ameri *et al.,* 2016). Furthermore, according to Abdel Hameed *et al.* (2020), the genetic diversity with a combined ISSR and SCoT showed that the use of both markers award a very efficient, cridable and more superior outcomes than the use of single markers.

ISSR marker have been utilized largely and effectively for the evolution of phylogenetic and fingerprinting. Hence, the present study aimed to use ISSR marker and morphological traits to analyze and find the genetic relationships among 66 (*F. carica*) accessions grown in Sulaymaniyah province, Kurdistan region, Iraq and it could be useful for future breeding programs.

MATERIAL AND METHODS

This study was conducted in the College of Agricultural Engineering Sciences, University of Sulaimani, in which 66 accessions of figs were collected during 2020–2021 from several geographical locations in Sulaymaniyah governorate, Kurdistan region-Iraq. They included wild types and cultivated figs of unknown identities. Newly grown fresh leaves were taken at the early growing season, kept in liquid nitrogen, for being used in the analysis of genetic polymorphism. Thereafter, during July-September, matured fruits and fully expanded leaves, each with 3 replicates and 10 pieces per a replicate, were randomly collected from the 66 different plants. All fruit samples were taken at the same level of physiological maturity, as visually determined. Measurements of current season shoot length (SL; cm), first internode diameter (SD; mm), leaf length (LL; cm), leaf width (LW; cm), leaf petiole length (LPL; cm) and leaf petiole diameter (LPD; mm) were taken by a measuring tape and digital Vernier calipers (0-150 mm; Model: DMV-SL05, WORKZONE, Germany). Also, the

pomological characteristics of fruit weight (FW; g) with a scale sensitive to 0.01 g (Precisa XB 2200 C, Precisa, UK), fruit length (FL; mm), fruit diameter (FD; mm), fruit stalk length (FSL; mm), fruit stalk diameter (FSD; mm) and ostiole diameter (OD; mm) with the same calipers, were taken. At time, the qualitative and quantitative characteristics of leaves and fruits were recorded according to the fig descriptors provided by the International Plant Genetic Resources Institute (IPGRI and CIHEAM, 2003).

ISSR analysis

ISSR marker was utilized to screen genetic diversity among the tested sixty-six fig cultivars and wild types, using fifteen primers for ISSR marker from previous studies (Sharifova *et al.,* 2017; Tahir *et al*., 2023). These primers were selected based on their effectiveness and reproducibility.

Table 1: List of ISSR Primers, their Nucleotide Sequences and Annealing Temperatures (T_m°C)

Name	Sequence (5'-3')	T_m˚C	Name	Sequence (5'-3')	T_m˚C
ISSR 1	AGACACACACACACACAT	50˚	**UBC 826**	ACACACACACACACACC	50˚
ISSR 11	ACACACACACACACACGG	50˚	**UBC 841**	GAGAGAGAGAGAGAGAYC	50˚
ISSR 12	AGAGAGAGAGAGAGAGCT	50˚	**UBC 845**	CTCTCTCTCTCTCTCTRG	50˚
UBC 808	AGAGAG AGAGAGAGAGC	50˚	**UBC 846**	CACACACACACACACART	50˚
UBC 810	GAGAGAGAGAGAGAGAT	50˚	**UBC 880**	GGAGAGGAGAGGAGA	50˚
UBC 813	CTCTCTCTCTCTCTCTT	50˚	**UBC 881**	GGG TGG GGT GGG GTG	54˚
UBC 815	CTCTCTCTCTCTCTCTG	50˚	**UBC891**	ACTACTACTTGTGTGTGTGTGTG	52˚
UBC 818	CACACACACACACACAG	52.8˚			

T_m°C; annealing temperatures

DNA Extraction

Total genomic DNAs were extracted from young healthy tissues of leaf samples of all cultivars and wild types of figs according to (Ahmed *et al.*, 2022), sodium dodecyl sulfate (SDS) method with a minor modification was used. The genome DNA extraction of fig leaves was achieved as following: young and fresh leaves were ground to a fine powder using liquid nitrogen. An adequate amount of leaf powder (about 300 mg) was used and transferred to a 2.0 mL Eppendorf tube. Each sample received 900 µL of lysis buffer with 10 µL of RNase. The contents were incubated at 64 °C for 70 minutes, and inverted 10 times during incubation, after that 300 µL of 5M potassium acetate (pH 6.5) was added to each sample, mixed well, and incubated in the refrigerator for at least 10 minutes. The supernatant was also transferred to a new tube and 800 µL of chloroform was added, the solution was mixed gently by inversion, the mixture was centrifuged at 14000 rpm for 17 minutes and the supernatant was taken. Then a volume of 1000 µL of binding buffer AW1 (2M guanidine thiocyanate,

75% ethanol) was added, the solution was mixed gently by inversion. The mixture was transferred to the spin column, centrifuged at 8000 rpm for 6 minutes, and then 500 µL of washing buffer AW2 (10 mM NaCl, 10 mM Tris-HCl pH 6.5, 80% ethanol) was added at 4000 rpm for 4 minutes. This step was repeated twice and then centrifuged at 12000 rpm for 6 minutes to dry the filtrate, the filtrate was then transferred to a new tube 1.5 mL, and then 100 µL of elution buffer was added to the filtrate, incubated at room temperature for 2 minutes, after that centrifuged at 9000 rpm for 5 minutes. The quality and quantity of extracted DNA were tested by Nano drop spectrophotometer (NanoPLUS-MAANLAB AB, SWEDEN); then it was used to estimate the obtained extracted DNA and its purity. All the DNA samples were stored at -20 °C for genotyping.

PCR amplification condition

PCR amplification reactions were accomplished within (25.0 µL) total volume containing 5.0 µL (80.0 ng) DNA template, 4.0 µL (10 µM) primer, 10.0 µL Master mix solution (AddStart Taq Master, Addbio,) and 6.0 µL deionized water (Mei *et al.,* 2015; Ahmed *et al.,* 2022). The PCR reaction was achieved in a Labnet Model: MultiGene OptiMax PCR machine as the following thermal cycle. As programed as initial denaturation step at 94 °C for 10 minutes, followed by 37 cycles of 1 minute at 94 °C, 1 minute at 50-54 °C (different for each primer of ISSR) and 2 minutes at 72 °C; then the final extension cycle for 10 minutes at 72 °C. All amplification products were separated in a 2% agarose gel containing 10 µL of ethidium bromide. The electrophoresis was performed in a 1X TBE buffer solution at 90 volts for 85 minutes. Finally, DNA fragment size was valued by using a 1 kb DNA ladder and gels were visualized and images were captured under UV light.

Statistical data analysis

The Principal Components Analysis (PCA) was used for the evaluation and description of the morphological data. Clustering was done by JMP Pro 16 software, ANOVA analysis using XLSTAT software version 2019 and Duncan's multiple range test were used to analyze differences among means ($P \leq 0.05$). PCA and Cluster Analysis were used to estimate the relationships among accessions and to determine the axes and the characters that significantly contribute in the variation. PCR-amplified ISSR marker fragments identified on gels were scored as absent (0) or present (1). The Power Marker version 3.25 program was utilized to measure major allele frequency (MAF). The Polymorphism Information Content (PIC) was measured by using the $PIC = 1-[f^2 + (1-f)^2]$ formula, where f is the marker frequency in the data set. Marker index (MI) was calculated by multiplying average of PIC for polymorphic band of each primer (De Riek *et al*., 2001; Ahmed *et al*., 2022). Investigation of population structure, a Bayesian model-based analysis was performed using

STRUCTURE 2.1 software (Pritchard *et al*., 2000) to detect genetic makeup and reveal the number of populations.

RESULTS AND DISCUSSIONS

The vegetative traits like (shoot length, first internode diameter of the current shoots, leaf length and width, as well as leaf petiole length and diameter) showed a high significant variability among fig accessions (Table 2). The highest value of current shoot length (79.953 cm) was recorded in accession (AC17), followed by (AC14) which recorded (77.187 cm), both of these were significantly superior to all other accessions except (AC55), where it was recorded (76.120 cm), however the lowest value (11.120cm) was noticed in the accession (AC44), which is a wild type of fig. Meanwhile, the highest value of the first internode diameter of the current shoots (15.563 mm) was recorded in (AC41), followed by AC53, AC58, AC50, AC64, AC55, and AC25 which recorded (15.020, 14.960, 14.947, 14.493, 14.323 and 14.220 mm), respectively. However, the lowest value (4.340 mm) for the first internode diameter of the current shoot was also recorded in AC12, which is a wild type. The highest values of leaf length and leaf width were recorded in the cultivated type AC20 with (28.183 and 28.480 cm), respectively, as well as the lowest values in both characteristics, length and width of leaves (10.91 and 9.813 cm), successively were recorded in the wild fig AC12. Furthermore, the highest value of leaf petiole length (13.397 cm) was recorded in AC17, followed by AC66 (13.050 cm), both accessions were significantly superior to all other accessions, but the lowest value of leaf petiole length (3.987 cm) was recorded in AC37. While the highest value of leaf petiole diameter (18.360 mm) was recorded in AC24, followed by AC53, AC27 and AC07 with the values (17.917, 17.903, and 17.710 mm), successively, while the lowest value (2.323 mm) was observed in AC52. The plant morphological results were acceptable and showed differences with the previous findings (Çalişkan and Polat, 2012). The shoot length was ranged between 7.8 (Sultani1) to 40 cm (Mor1). Leaf length and width were varied between (16.4 to 27.6 cm) and (16.0 to 23.5 cm), respectively. These results are in harmony with those found by (Simsek *et al.*, 2017; Khodaee *et al.*, 2021). The reasons for such variations may return to the variations in genetic characteristics, environmental conditions (climatic and soil conditions), and agricultural techniques (pruning, irrigation, plowing and fertilization). In this study, plants have varied ages, and agriculture management. (Chithiraichelvan *et al*., 2017), revealed that pruning and plant age have a significant impact on growth habit and fruit characteristics.

Table 2**:** Plant Morphological Traits of Fig Accessions Used in the Present Study

Accessions	SL	SD	LL	LW	LPL	LPD
AC01	19.700 r-w	6.280 x-c	18.433 f-o	11.000 vw	6.000 t-ad	3.800 h-n
AC02	25.933 mno	4.663 aa-c	13.800 v-z	13.067 r-v	5.887t-ae	3.950 h-l
AC03	26.733 mn	9.700 k-t	16.400 l-v	15.733 iq	6.743 o-x	4.203 g-k
AC04	23.233 n-s	11.077 g-m	19.920 c-h	22.200 b	7.170 l-u	5.527 g
AC05	15.840 v-z	7.920 p-x	21.107 b-f	19.873 cd	11.667 b	4.087 h-l
AC06	16.267 u-y	4.893 aa-c	17.733 h-q	13.933 o-u	4.633 aa-f	3.320 i-n
AC07	13.147 xyz	12.250 e-j	13.537 w-z	17.710 d-j	10.063 cde	17.710 a
AC08	16.010 v-y	6.793 v-a	19.820 c-i	13.923 o-u	11.207 bc	3.930 h-l
AC09	29.010 klm	10.773 g-o	18.410 f-o	19.103 cde	9.260 d-i	3.593 h-n
AC10	37.300 g-j	9.217 m-u	21.197 b-e	17.507 d-k	4.730 ac-f	4.840 gh
AC11	15.633 v-z	7.787 q-x	15.537 p-y	17.847 d-j	6.190 s-ab	4.343 g-j
AC12	12.597 yz	4.340 aa-c	10.910 aa	9.813 w	5.113 z-af	2.807 k-n
AC13	20.127 p-v	9.983 k-q	14.263 t-z	14.933 l-s	7.080 m-v	11.627 d
AC14	77.187 a	7.843 p-x	15.007 r-y	13.963 p-u	8.017 h-o	3.717 h-n
AC15	16.907 u-y	5.403 z-c	17-057 j-s	13.580 q-u	6.023 t-ad	2.903 j-n
AC16	37.243 g-j	8.660 o-v	21.537 bcd	16.957 e-l	8.887 e-j	8.460 f
AC17	79.953 a	7.923 p-x	20.553 c-g	13.643 q-u	13.397 a	13.643 c
AC18	38.187 ghi	7.673 s-y	15.533 p-y	14.447 l-t	5.713 v-ae	3.570 h-n
AC19	72.047 b	9.700 k-t	20.120 c-h	17.683 d-j	9.793 def	3.613 h-n
AC20	17.773 t-x	9.863 k-s	28.183 a	28.480 a	8.827 e-j	4.337 g-j
AC21	41.424f g	13.317 b-f	18.443 f-n	17.960 d-j	8.760 e-j	3.423 h-n
AC22	60.027 c	8.797 n-v	20.363 c-h	18.227 d-i	8.813 e-j	3.337 i-n
AC23	34.273 ij	11.563 f-l	14.967 r-y	13.853 p-u	9.677 d-g	2.413 mn
AC24	55.723 cd	7.793 q-x	17.200 i-s	18.360 c-h	10.473 bcd	18.360 a
AC25	17.447 u-y	14.220 a-e	14.480 s-y	17.957 d-j	5.970 t-ad	3.720 h-n
AC26	24.833 m-q	7.707 r-y	15.323 q-y	16.343 f-p	6.633 o-y	3.520 h-n
AC27	17.463 u-y	12.337 d-j	18.203 g-p	17.903 d-j	8.457 f-m	17.903 a
AC28	19.977 q-w	6.337 x-c	13.527 w-z	12.783 s-v	6.493 q-z	3.407 h-n
AC29	22.570 n-t	5.660 y-c	20.063 c-h	16.593 e-n	7.943 h-p	2.643 lmn
AC30	16.267 u-y	8.720 o-v	14.687 s-y	16.127 g-q	8.580 f-l	16.127 b
AC31	43.713 f	7.593 t-y	17.840 g-q	15.517 j-r	8.480 f-m	4.680 ghi
AC32	33.173 jk	9.600 k-t	19.217 d-k	17.563 d-k	6.120 t-ac	3.023 j-n
AC33	24.360 m-r	10.173 j-o	17.677 h-r	16.777 e-m	6.630 o-y	3.273 i-n
AC34	18.160 t-x	10.510 h-o	18.590 e-m	18.373 c-h	5.713 v-ae	4.320 g-j
AC35	21.127 o-v	10.670 h-o	20.223 c-h	20.630 bc	8.707 e-k	3.630 h-n
AC36	24.427 m-r	8.790 n-v	18.820 e-m	17.743 d-j	4.773 ab-f	3.850 h-m
AC37	39.110 fgh	11.550 f-l	16.830 k-t	16.037 h-q	3.987 aa-f	3.733 h-n
AC38	17.383 u-y	9.850 k-s	18.830 e-m	19.050 cde	7.563 j-s	3.643 h-n

Continue table 2.

Accessions	SL	SD	LL	LW	LPL	LPD
AC39	39.767 fg	10.770 g-o	16.810 k-t	11.888 uvw	9.493 d-g	3.307 i-n
AC40	16.710 u-y	8.697 o-v	16.133 m-w	12.890 s-v	5.453 x-ae	3.110 j-n
AC41	54.400 d	15.563 a	23.503 b	18.173 d-i	8.457 f-m	3.377 h-n
AC42	28.303 lm	9.933 k-r	15.730 o-x	14.763 l-s	8.880 e-j	4.003 h-l
AC43	34.637 hij	9.397 l-t	21.540 bcd	15.050 k-s	7.823 i-q	3.280 i-n
AC44	11.120 z	6.477 w-ab	13.743 v-z	12.727 s-v	5.603 w-ae	2.580 lmn
AC45	28.360 lm	11.803 f-k	18.157 g-p	18.807 c-f	7.303 k-t	3.647 h-n
AC46	25.993 mno	10.043 k-p	15.790 n-x	16.440 f-o	6.580 p-y	3.013 j-n
AC47	48.673 e	10.220 i-o	14.970 r-y	14.310 m-u	8.737 e-j	2.707 k-n
AC48	29.070 kl	9.427 l-t	11.677 zaa	10.347 w	5.443 x-ae	10.347 e
AC49	28.990 klm	11.147 g-m	22.140 bc	18.667 c-g	7.627 j-r	3.567 h-n
AC50	55.373 d	14.947 abc	19.553 c-j	16.023 h-q	6.840 o-x	3.717 h-n
AC51	25.373 mno	7.057 u-z	21.123 b-f	14.870 l-s	8.807 e-j	2.770 k-n
AC52	21.307 o-v	8.580 o-w	13.940 u-z	11.037 vw	4.533 aa-f	2.323 n
AC53	36.993 g-j	15.020 ab	15.307 q-y	17.917 d-j	8.300 g-n	17.917 a
AC54	55.447 d	12.937c-g	17.807 h-q	14.430 l-t	6.937 n-w	2.403 mn
AC55	76.120 ab	14.323 a-d	18.630 e-m	16.957 e-l	9.300 d-h	3.560 h-n
AC56	52.360 de	12.407 d-i	14.623 s-y	13.627 q-u	5.967 t-ad	13.627 c
AC57	14.987 w-z	9.820 k-s	12.897 y-a	13.790 q-u	5.740 u-ae	13.790 c
AC58	55.050 d	14.960 abc	18.923 d-l	14.687 l-s	6.647 o-y	3.347 h-n
AC59	54.963 d	11.813 g-k	15.010 r-y	16.947 e-l	4.953 aa-f	3.367 h-n
AC60	15.053 v-z	7.920 p-x	13.170 x-a	12.117 t-w	4.613 aa-f	3.147 j-n
AC61	55.943 cd	11.007 g-n	16.347 l-v	16.667 e-n	7.887 h-q	3.297 i-n
AC62	25.017 m-p	9.743 k-t	16.697 k-t	14.167 n-u	5.990 t-ad	4.023 h-l
AC63	19.037 s-w	10.997 g-n	16.637 k-u	16.687 e-n	5.247 y-af	3.957 h-l
AC64	40.767 fg	14.493 abc	20.063 c-h	16.440 f-o	7.593 j-s	3.340 i-n
AC65	28.497 klm	11.503 f-l	13.600 w-z	14.507 l-t	6.313 r-aa	3.630 h-n
AC66	33.030 jkl	12.457 d-h	19.027 d-l	16.037 h-q	13.050 a	3.420 h-n

Means with different letters in the same column are differ significantly (P≤0.05).

SL: shoot length (cm), SD: shoot diameter (mm), LL: leaf length (cm), LW: leaf width (cm), LPL: leaf petiole length (cm), LPD: leaf petiole diameter (mm) and R^2: correlation coefficient.

Fig accessions showed highly significant variances in all the fruit characteristics. As shown in Tables 3 and 4, the highest value of average fruit weight (63.447 g) was recorded in AC03 which is the yellow-skinned fruit, oblate shape, and of cultivar types that are significantly superior to all the other fig accessions. AC19 and AC22 had recorded (60.924 and 58.732 g), respectively. However, the lowest value of average fruit weight (4.483 g) was recorded in AC37, the dark violet-skinned and wild type fig. The fruit appearance is influential on the fig fruits consumption. Hence, small fruits are

generally used for canning, whereas big ones are consumed freshly. Besides, the fruits reflect the proper conservation of the trees (Tamboli *et al*., 2015; Hssaini *et al*., 2020). This result is similar to those obtained by (Pereira *et al*., 2017), in which fruit weight was ranged from 27.2 to 56.8 g. The highest fruit length value (46.960 cm) was recorded in AC22, a yellow skin fruit, oblate and a cultivated fig, this was significantly superior to all the other fig accessions except AC03 which recorded (45.633 cm), followed by AC19 and AC53 with (44.383 and 41.760 cm), respectively. Nevertheless, the lowest value (14.770 cm) was recorded in AC37, a dark violet-skinned and wild type fig. Meanwhile, the highest value of fruit diameter (60.420 cm) was recorded in AC54, which has a dark-violet fruit skin, an oblate fruit shape, and is a cultivar fig; AC19 showed the average value (57.467 cm). However, the lowest fruit diameter (18.497 cm) was recorded in AC12, dark-violet and wild fig. Furthermore, the highest value of fruit stalk length (29.887 mm) was recorded in AC58, it is a fruit of brown skin, oblate fruit shape and cultivar fig which was significantly superior to all the other fig accessions, followed by AC64 and AC03 which recorded (21.797 and 21.333 mm), successively. Whereas the lowest value (2.373 mm) for the mentioned trait was recorded in AC08, it is the dark-violet skin fruit, oblate fruit shape, and wild fig. The highest value of fruit stalk diameter (10.433mm) was recorded in AC58 and was significantly superior to all the other ACs. It has a brown fruit skin, an oblate fruit shape, and is a cultivar fig, and AC20 recorded (7.553 mm). Whereas the lowest value (2.533 mm) for the same characteristic was recorded for AC25, it is the yellow fruit skin, oblate fruit shape, and cultivar fig. The highest value of ostiole diameter (8.717 mm) was shown in AC14, it is the light red fruit skin, oblate fruit shape, and cultivar fig, which was significantly superior to all the other ACs, followed by AC09, AC13, AC27, AC49 and AC26 which recorded (8.040, 7.840, 7.707, 7.690 and 7.660 mm), successively. Even though, the lowest value (2.557 mm) was recorded in AC30, which is the green fruit skin, globose fruit shape and cultivar fig. These results may refer to the existence of a wide range of genetic diversity among the fig accessions at the bases of morphological and pomological characteristics. In the past, many studies were done on plants morphological and pomological characteristics, this study had similar results and/or partial differences with the previous researchers like (Simsek *et al.,* 2017; Ali 2019, Hssaini *et al.,* 2020).

Table 3: Fruit Traits of the Fig Accessions

Accessions	FW	FL	FT	FSL	FSD	OD
AC01	23.626 st	23.187 y-ab	36.367 r-u	12.417 j	3.183 t-z	6.107 f-j
AC02	26.296 p	27.003 u-x	42.613 i-l	2.600 aa-d	4.007 m-s	5.577 h-m
AC03	63.447 a	45.633 ab	55.833 bc	21.333 b	4.367 k-q	6.433 efg
AC04	18.720 xy	29.323 p-v	36.767 q-t	8.553 rst	4.983 f-l	3.787 t-x
AC05	31.885 lm	31.590 m-q	31.487 vw	15.270 gh	4.700 h-m	6.187 e-i
AC06	18.189 yz	28.367 r-w	33.330 uv	17.250 d	3.917 n-t	5.633 h-l
AC07	38.792 i	32.630 k-o	49.727 efg	6.157 y	4.490 j-o	6.143 f-j
AC08	14.916 aab	20.710 aab	25.340 zaa	2.373 aa-d	5.133 f-k	3.313 x
AC09	24.203 rs	32.200 l-p	43.047 ijk	2.423 aa-d	5.223 f-j	8.040 b
AC10	18.503 xy	25.507 wxy	38.957 m-r	10.030 n-q	4.940 f-l	3.150 xy
AC11	25.781 pq	24.543 xyz	39.757 l-q	7.350 t-y	5.380 e-i	5.623 h-l
AC12	13.910 aab	17.530 aa-c	18.497 aa-c	11.860 jkl	3.293 s-y	3.893 s-x
AC13	26.256 p	29.643 p-u	41.550 j-m	4.467 z-aa	3.710 p-u	7.840 bc
AC14	23.979 st	30.133 o-t	38.667 m-r	7.330 t-y	3.593 r-w	8.717 a
AC15	10.559 aa-d	21.687 aa-b	28.453 xy	3.650 aa-b	2.967 u-aa	5.600 h-m
AC16	20.549 vw	30.110 o-t	33.383 uv	8.513 r-u	4.760 h-m	4.570 o-s
AC17	16.984 zaa	29.087 q-v	37.767 o-r	9.040 qrs	3.690 p-u	5.940 f-k
AC18	22.510 tu	29.030 q-v	42.440 i-l	4.867 z	4.427 k-p	4.553 o-s
AC19	60.924 b	44.383 b	57.467 b	11.113 k-n	5.023 f-l	6.487 ef
AC20	23.118 stu	32.293 l-p	38.670 m-r	12.570 j	7.553 b	5.100 l-q
AC21	30.280 no	31.827 l-q	40.397 k-p	10.430 m-p	5.550 d-g	6.513 ef
AC22	58.736 c	46.960 a	56.467 bc	15.957 e-h	6.140 cd	5.397 j-n
AC23	51.249 de	38.427 def	54.390 cd	6.203 y	5.443 d-h	5.223 k-p
AC24	43.407 g	39.267 cde	52.623 de	9.643 o-r	4.390 k-p	6.420 efg
AC25	18.539 xy	27.360 t-x	33.920 tuv	12.227 jkl	2.533 zaa	4.910 l-r
AC26	25.543 pqr	37.207 e-h	47.837 gh	16.970 de	4.447 k-p	7.660 bc
AC27	25.925 pq	30.453 n-s	22.730 aa-b	5.033 z	2.580 y-aa	7.707 bc
AC28	26.229 p	30.667 n-s	44.873 hi	7.747 t-w	3.390 s-x	3.823 s-x
AC29	29.012 o	33.320 j-m	41.113 j-n	10.823 l-o	4.927 f-l	6.353 e-h
AC30	11.944 aa-c	26.653 vwx	27.870 xyz	11.583 j-m	2.880 v-aa	2.557 y
AC31	16.403 aa	28.277 r-w	34.127 s-v	6.570 wxy	3.447 s-x	4.370 q-u
AC32	19.017 xy	24.933 xyz	37.233 pqr	9.727 o-r	2.830 w-aa	4.517 o-t
AC33	26.975 p	32.707 k-o	40.923 j-o	10.770 l-o	3.513 s-w	6.430 efg
AC34	21.930 uv	27.093 u-x	37.123 qrs	13.723 i	4.904 f-l	5.210 k-p
AC35	41.101 h	37.473 e-h	46.923 gh	12.230 jk	3.903 n-t	4.727 n-r
AC36	14.594 aa-b	24.740 xyz	28.040 xyz	7.240 u-y	3.560 r-w	3.640 u-x

Continue table 3.

Accessions	FW	FL	FT	FSL	FSD	OD
AC37	4.483 aa-f	14.770 aa-d	21.440 aa-b	3.613 aa-c	5.367 e-i	3.877 s-x
AC38	25.960 pq	25.823 wxy	47.003 gh	4.697 z-aa	4.460 j-p	6.430 efg
AC39	16.605 aa	22.173 z-ab	39.810 l-q	10.760 l-o	3.563 r-w	3.597 vwx
AC40	19.694 wx	27.160 t-x	34.087 s-v	9.397 pqr	3.777 o-t	4.443 p-t
AC41	24.490 pqr	32.680 k-o	38.717 m-r	2.877 aa-d	4.807 g-l	3.570 vwx
AC42	30.992 mn	29.797 o-u	47.477 gh	6.383 xy	4.380 k-p	4.593 o-s
AC43	11.148 aa-d	20.940 aa-b	29.017 wx	10.670 l-o	3.440 s-x	4.807 n-r
AC44	7.415 aa-e	21.547 aa-b	25.810 yz	7.620 t-x	2.743 x-aa	3.517 x
AC45	32.663 l	29.617 p-v	50.027 efg	7.977 s-v	5.443 d-h	6.283 e-h
AC46	37.582 ij	34.680 h-l	43.753 ij	19.563 c	2.910 v-aa	6.507 ef
AC47	20.643 vw	32.280 l-p	33.067 v	16.667 def	3.873 o-t	3.420 x
AC48	10.458 aa-d	27.060 u-x	27.567 xyz	14.953 h	2.347 aa	3.547 wx
AC49	51.938 de	40.507 cd	48.937 fg	17.417 d	4.307 l-r	7.690 bc
AC50	24.549 qrs	32.693 k-o	38.303 m-r	11.693 j-m	3.410 s-x	4.290 r-w
AC51	31.422 lmn	32.210 l-p	38.933 m-r	11.677 j-m	5.650 def	4.850 m-r
AC52	48.273 f	36.590 e-i	47.567 gh	17.517 d	4.427 k-p	4.317 q-v
AC53	52.482 d	41.760 c	48.920 fg	13.777 i	4.517 j-o	6.437 efg
AC54	34.780 k	36.003 f-j	60.420 a	15.723 fgh	3.410 s-x	6.160 e-j
AC55	48.091 f	29.450 p-v	51.443 ef	16.300 d-g	3.823 o-t	6.313 e-h
AC56	51.624 de	38.413 def	49.787 efg	15.387 gh	6.593 c	5.670 g-l
AC57	23.725 st	28.103 s-w	38.203 n-r	11.950 jkl	4.650 i-n	7.243 cd
AC58	28.939 o	23.743 x-aa	37.007 qrs	29.887 a	10.433 a	6.947 de
AC59	50.592 e	36.793 e-i	49.813 efg	20.223 c	6.030 cde	4.717 n-r
AC60	31.259 lmn	27.980 s-w	41.057 j-n	7.630 t-x	4.740 h-m	4.753 n-r
AC61	51.175 de	37.643 efg	49.760 efg	17.520 d	5.570 d-g	6.200 e-h
AC62	32.217 lm	31.190 m-r	47.447 gh	6.813 v-y	4.013 m-s	5.423 i-n
AC63	36.480 j	35.817 f-j	42.500 i-l	10.470 m-p	6.400 c	4.563 o-s
AC64	34.399 k	35.340 g-k	40.430 k-p	21.797 b	3.613 q-v	6.623 def
AC65	51.331 de	34.070 i-m	49.753 efg	15.613 fgh	5.290 e-i	5.297 k-o
AC66	42.307 gh	29.993 o-u	43.503 ijk	7.623 t-x	3.420 s-x	4.713 n-r

Mean with different letters in the same column differ significantly (P≤0.05).

AC: accession, FW: fruit weight (g), FL: fruit length (mm), FT: fruit thickness (mm), FSL: fruit stalk length (mm), FSD: fruit stalk diameter (mm), OD: ostiole diameter (mm) and R^2: correlation coefficient.

Table 4: Pomological Traits of the Fig Accessions

Accessions	Fruit skin color	Pulp color	Fruit shape	Beginning of fruit ripened	Germplasm types
AC01	Light-Violet	Maroon	Oblate	Very early	Cultivar
AC02	Yellow	Maroon	Oblate	Very early	Cultivar
AC03	Yellow	Maroon	Oblate	Early	Cultivar
AC04	Yellow	Amber	Oblate	Early	Cultivar
AC05	Yellow	Red	Globose	Mid-season	Cultivar
AC06	Light-Violet	Maroon	Globose	Early	Wild
AC07	Yellow	Amber	Oblate	Very later	Cultivar
AC08	Dark-Violet	maroon	Oblate	Very later	Wild
AC09	Light-Red	pink	Oblate	Mid-season	Cultivar
AC10	Dark-Red	pink	Oblate	Mid-season	Cultivar
AC11	Yellow	Maroon	Oblate	Mid-season	Cultivar
AC12	Dark-Violet	Marron	Globose	-----	Wild-Capri fig
AC13	Light-Red	Amber	Oblate	Mid-season	Cultivar
AC14	Light-Red	pink	Oblate	Later	Cultivar
AC15	Dark-Violet	Marron	Oblate	Very later	Wild
AC16	Yellow	Maroon	Globose	Mid-season	Cultivar
AC17	Yellow green	Maroon	Oblate	Mid-season	Cultivar
AC18	Yellow green	Amber	Oblate	Mid-season	Cultivar
AC19	Yellow	Maroon	Oblate	Mid-season	Cultivar
AC20	Yellow green	Red	Globose	Mid-season	Cultivar
AC21	Bright-Yellow	Red	Oblate	Mid-season	Cultivar
AC22	Yellow	Maroon	Oblate	Early	Cultivar
AC23	Dark-Violet	Amber	Oblate	Mid-season	Cultivar
AC24	Green	Amber	Oblate	Mid-season	Cultivar
AC25	Yellow	Maroon	Oblate	Mid-season	Cultivar
AC26	Dark-Violet	Amber	Oblate	Later	Cultivar
AC27	Light-Green	pink	oblong	Mid-season	Cultivar
AC28	Yellow	Maroon	Oblate	Mid-season	Cultivar

Continue table 4.

Accessions	Fruit skin color	Pulp color	Fruit shape	Beginning of fruit ripened	Germplasm types
AC29	Dark-Violet	Maroon	Oblate	Mid-season	Cultivar
AC30	Green	maroon	Globose	Later	Cultivar
AC31	Yellow	Amber	Oblate	Mid-season	Cultivar
AC32	Dark-Violet	Yellow	Oblate	Mid-season	Cultivar
AC33	Yellow	Maroon.	Oblate	Later	Cultivar
AC34	Red	pink	Oblate	Later	Cultivar
AC35	Yellow	Maroon	Oblate	Later	Cultivar
AC36	Violet	Amber	Globose	Mid-season	Cultivar
AC37	Dark-Violet	Yellow	Oblate	----	Wild-Capri fig
AC38	Yellow	amber	Oblate	Mid-season	Cultivar
AC39	Red	pink	Oblate	Mid-season	Cultivar
AC40	Yellow	Maroon	Oblate	Mid-season	Cultivar
AC41	Dark-Violet	Yellow	Globose	Mid-season	Cultivar
AC42	Yellow	maroon	Oblate	Mid-season	Cultivar
AC43	Dark-Violet	Maroon	Oblate	Early	Cultivar
AC44	Dark-Violet	maroon	Globose	---------	Wild-Capri fig
AC45	Yellow green	Amber	Oblate	Mid-season	Cultivar
AC46	Light-Brown	Maroon	Oblate	Mid-season	Cultivar
AC47	Brown	Amber	Globose	Mid-season	Cultivar
AC48	Light-Green	Amber	Globose	Mid-season	Cultivar
AC49	Brown	Amber	Oblate	Mid-season	Cultivar
AC50	Yellow	Maroon	Globose	Mid-season	Cultivar
AC51	Brown	Amber	Oblate	Mid-season	Cultivar
AC52	Violet	Maroon	Oblate	Mid-season	Cultivar
AC53	Green	Maroon	Globose	Mid-season	Cultivar
AC54	Dark-Violet	Maroon	Oblate	Mid-season	Cultivar
AC55	Yellow	Amber	Oblate	Mid-season	Cultivar
AC56	Light-Green	Maroon	Oblate	Mid-season	Cultivar

Continue table 4.

Accessions	Fruit skin color	Pulp color	Fruit shape	Beginning of fruit ripened	Germplasm types
AC57	Green	maroon	Oblate	Mid-season	Cultivar
AC58	Brown	Amber	Oblate	Mid-season	Cultivar
AC59	Yellow	Amber	Oblate	Mid-season	Cultivar
AC60	Yellow	Maroon	Oblate	Mid-season	Cultivar
AC61	Yellow	Amber	Oblate	Mid-season	Cultivar
AC62	Yellow	Amber	Oblate	Mid-season	Cultivar
AC63	Yellow	Amber	Globose	Mid-season	Cultivar
AC64	Dark-Violet	Amber	Globose	Mid-season	Cultivar
AC65	Yellow	Maroon	Oblate	Later	Cultivar
AC66	Yellow	Pink	Oblate	Mid-season	Cultivar

Very early (<20 July), Early (20-31 July), Mid-season (1-15 August), Late (15-31 August), Very late (>31 August). Fruit shape [index (I) = (width/length)], Oblong (I < 0.9), Globose (I = 0.9-1.1), Oblate (I > 1.1).

Principal component analysis (PCA) is the most informative graphical technique used for representing and evaluating complex and large datasets. The form of variability in fig accessions was assessed using principal component analysis based on the correlation between the characteristics and the clusters to assess the variety of the accessions and their relationships with the observed characteristics. The vectors are clarified by the more investigated features, which interact positively and negatively. The vector length of the trait indicates the magnitude of its influence on the dependent character and the angle between vectors produced from the middle point determines the degrees of correlation between the descriptors. However, the vector direction refers to the positive and negative relationship between the descriptors (Girgel, 2021). PCA referred that the first twelve components with eigenvalues ranging from 0.10 to 4.08 were significant in elucidating the variation among the 66 accessions investigated and cumulatively accounted for 100% of the total phenotypic variation (Table 5). Although, results determined that the first two principal components described 49.15% of the total quality variance. F1 counted for 33.99% of total variance with the eigenvalue (4.08), whereas F2 counted for 15.16% of total variance with an eigenvalue (1.82). Though, the bi plot showed that both (LL and FWT) characters were the longest among them, indicating that the two mentioned traits had more effects on the variation (Figure 1), however; (LPD) was the shortest among them denoting to the less effect on the variation. Furthermore, each (LL, LW, LPL, FSD, SD and SL) have the same directions on the above part of the figure. They had positive correlations among them, however each (FL, FT, FWT, OD, FSL, and LPD) have the same directions and positive correlations on the under

part of figure. While the relationship between the two groups were negatively correlated. The angle between the two vectors indicates to the power of correlations such as (LW with LPL, SL with SD, FT with FWT, FWT with OD, OD with FSL or FSL with LDP) having heavy acute angles and strong correlations. However, the right-angle feature indicates to no correlation between two vectors such as (LL with FWT or LW with FSL), whereas the obtuse angle feature indicates to negative and weak correlations such as the angle between (LL with LPD). According the ratio of correlation among the fig accessions, it was produced about five accession groups. The two groups (Gr III and Gr IV) included the most of fig accessions and nearly took the center of the variation (Figure 1), however, the other groups (Gr I, Gr II and Gr V), were far away in the center and had more effect on the variation. As well as, a number of accessions (52, 48, 20 and 12), were getting outside of the mentioned groups and distant of the center, respectively, with a more effectiveness on the variation. All fig accessions mentioned above had globose fruit shapes, AC12 was wild type of fig germplasm; it had less weight and fruit length and width as well as violet skin color. The results are near with other researchers. Bhavana *et al*. (2019) proposed that the first three principal components are often the most significant in reflecting the variation patterns among accessions, and the characters related with these are more valuable in differentiation among the accessions. The results are in conformity with those obtained by Manyasa *et al*. (2009) who gained similar results.

Table 5: Eigenvalues, Variance, Cumulative Variance and Principal Component Scores of the 12 Components Based on the 12-Plant Morphological and Fruit Traits among 66 Fig Accessions

Variable	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	F12
SL	0.27	0.05	0.08	-0.70	-0.05	0.28	-0.12	-0.14	-0.49	-0.26	-0.05	0.06
SD	0.34	0.04	-0.10	-0.29	0.35	-0.23	-0.18	-0.49	0.53	0.19	-0.16	0.04
LL	0.14	0.64	-0.12	0.00	-0.17	0.07	0.18	-0.07	-0.19	0.63	0.20	0.06
LW	0.21	0.52	-0.03	0.40	0.12	-0.18	0.06	-0.26	-0.14	-0.61	-0.03	0.10
LPL	0.16	0.36	0.50	-0.25	-0.17	0.01	0.15	0.46	0.49	-0.17	-0.02	-0.08
LPD	0.03	-0.04	0.62	0.12	0.67	0.00	-0.02	0.04	-0.26	0.19	0.21	0.03
FWT	0.43	-0.23	0.03	0.13	-0.15	-0.16	-0.01	0.24	-0.06	0.09	-0.10	0.79
FL	0.43	-0.14	0.12	0.16	-0.13	-0.25	0.15	0.05	-0.26	0.17	-0.55	-0.51
FT	0.41	-0.20	0.03	0.07	-0.27	-0.21	-0.26	-0.04	0.02	-0.06	0.72	-0.28
FSL	0.25	-0.17	-0.37	-0.12	0.34	0.07	0.73	0.18	0.05	-0.11	0.23	-0.07
FSD	0.24	0.16	-0.39	0.12	0.33	0.29	-0.51	0.52	0.01	0.01	-0.09	-0.14
OD	0.26	-0.14	0.18	0.33	-0.13	0.78	0.08	-0.31	0.21	0.01	-0.05	-0.01
Eigenvalue	4.08	1.82	1.41	1.04	0.89	0.72	0.65	0.58	0.37	0.20	0.14	0.10
Variability (%)	33.99	15.16	11.79	8.68	7.39	5.99	5.39	4.83	3.04	1.65	1.21	0.87
Cumulative %	33.99	49.15	60.94	69.62	77.02	83.00	88.40	93.23	96.27	97.92	99.13	100.00

Note: PCA principal component analysis, SL: shoot length, SD: Shoot Diameter, LL: leaf length, LW: leaf width, LPL: leaf petiole length, LPD: leaf petiole diameter, FWT: fruit weight, FL: fruit length, FT: fruit thickness, FSL: fruit stalk length, FSD: fruit stalk diameter, OD: ostiole diameter.

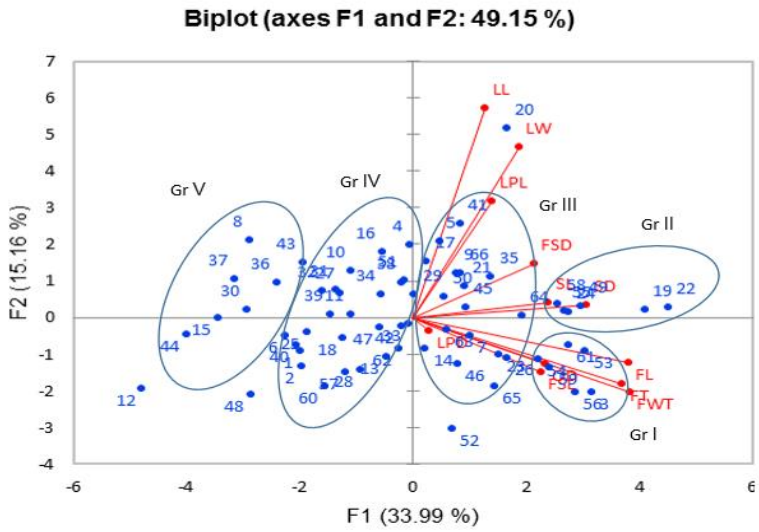


Figure 1. Principal Component Analysis (PCA) biplot clarifying the distribution of morphological and pomological traits in the first principal component and second principal component.

LL: leaf length (cm), LW: Leaf width (cm), LPL: Leaf petiole length (mm), FSL: Fruit stalk diameter (mm), SL: Shoot length (cm), SD: Shoot Diameter (mm), FL: Fruit length (mm), FT: Fruit thickness (mm), FWT: Fruit weight (g), OD: Ostiole diameter (mm), FSL: Fruit stalk length (mm), LPD: Leaf petiole diameter (cm), Numbers (1-66) fig accessions.

Cluster analysis using the unweighted pair group method with arithmetic mean (UPGMA) created on morphological distance analysis showed that the 66 accessions were assembled into six main large phenotypically correlated clusters (Figure 2). Finer grouping inside some of the main clusters was also instituted. So, some were divided into sub-clusters to better define the variability. The first cluster (I) is dark blue including 10 accessions (AC01, AC06, AC02, AC18, AC28, AC40, AC60, AC12, AC15, AC44), these accessions had more correlation among them compared to the accessions existing in the next cluster. Then the red cluster (II) had contained 13 accessions (AC08, AC10, AC32, AC36, AC16, AC31, AC43, AC37, AC25, AC39, AC47, AC30 and AC48). In addition, the blue cluster (III) consists of 15 accessions (AC03, AC52, AC65, AC56, AC59, AC61, AC19, AC22, AC26, AC33, AC46, AC49, AC64, AC54 and AC55). However, the yellow cluster (IV) includes only one accession (AC58) that was completely different from all other selected cultivars and wild types. Moreover, as inferred from the morphological data matrix, the gray cluster (V) comprises of 19 accessions (AC04, AC35, AC11, AC34, AC63, AC09, AC38, AC45, AC21, AC05, AC29, AC51, AC23, AC42, AC62, AC66, AC41, AC50, AC20). Lastly, the purple cluster (VI) confines the rest of 8.0 accessions (AC07, AC53, AC24, AC13, AC57, AC27, AC14, and AC17). These results are close to other previous records in the fig studies carried out independently by each Caliskan *et al.* (2018) and Abdelsalam *et al.* (2019), who all confirmed the existence of a high diversity in plant morphological and pomological related traits. This could be valuable as an efficient marker system to differentiate among fig genotypes and grouping fig accessions. In classification researches, particularly with a large number of accessions, traits that are dependent on environmental conditions,

such as morphological and fruit quality features, were not instituted to be very useful (Baraket *et al*., 2011).

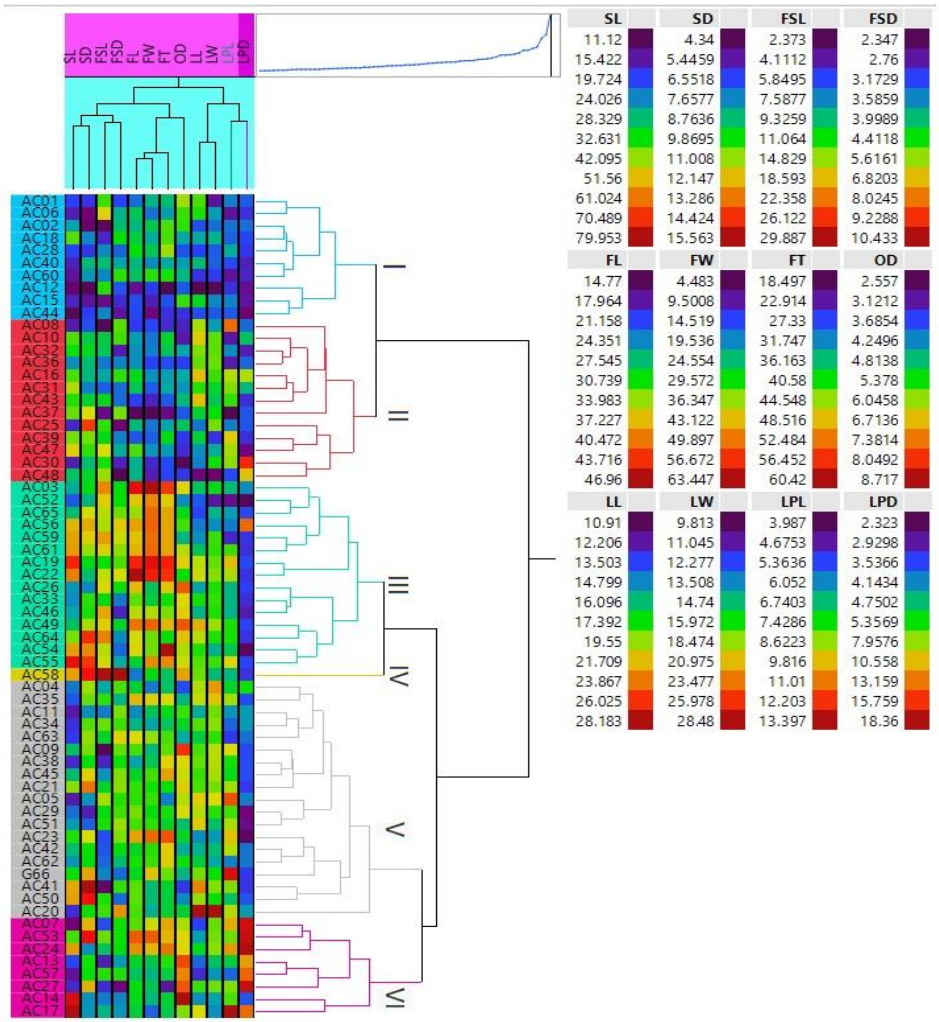


Figure 2. Dendrogram of hierarchical clustering created by JMP Pro based on 12 plant morphological and pomological traits attributed to 66 accessions.

SL: Shoot length (cm), SD: Shoot diameter (mm), FSL: Fruit stalk length (mm), FSD: Fruit stalk diameter (cm), FL Fruit length (mm), FT: Fruit thickness (mm), FW: Fruit weight, OD: Ostiole diameter (mm), LL: leaf length (cm), LW: Leaf width (cm), LPL: Leaf petiole length (cm) and LPD: Leaf petiole dimeter (mm).

Fifteen ISSR primers were selected to examine the genetic variability among 66 fig germplasms. A total of (197) bands were formed (Table 6). The average percentage of polymorphism was shown to be 100% for all of the primers. The number of polymorphic bands differed between 9 bands (UBC826) and 18 bands (ISSR12), with a mean of 13 bands per primer. The highest PIC value was 0.979 (ISSR12 and UBC891) and the lowest value was 0.878 (UBC826), with a mean of 0.950 per primer. Furthermore, the highest versity (0.980) was found in UBC891 and the lowest value was (0.883) in UBC826, with an average of (0.952). Moreover, the highest allele frequency (0.303) was found in UBC826 and the lowest value (0.045) was recorded in (ISSR12, UBC881, and

UBC891), with an average of (0.128). The number of alleles per locus was ranged from 35 alleles in ISSR1 to 56 alleles in (ISSR12 and UBC891), with a mean of 43.066 alleles per locus. These results are close to those found by Rout and Aparajita (2009) who detected 116 bands, among which 106 (91.3%) of them were polymorphic, when they tested 23 fig accessions with 5 ISSR primers. In addition, the average number of polymorphic bands per primer was 21 with a range from 15 to 31. Also, 13 ISSR primers were determined to test 19 fig varieties and produced 46 polymorphic bands ranging from 0.00% polymorphism in UBC826 to 100% polymorphism in (UBC815 and UBC817), with an average of 3.5% polymorphic bands per primer (Ikegami *et al.,* 2009). In another study Abdelsalam *et al.* (2019) that analyzed the polymorphism among 21 fig accessions, while investigated by 12 ISSR primers, they found that the polymorphism had differed between 50% for UBC807 and 100% for UBC817. As well as the bands number ranged from 4.0 bands in (UBC808, UBC810 and UBC816) to 8.0 bands in UBC823. The highest allele diversity value 0.961 was noticed in UBC810 and the lowest value 0.683 was given by UBC808. Moreover, the PIC values were ranged from 66.7% in UBC808 to 90.9% in UBC810. Furthermore, the genetic polymorphism of 39 germplasms among lemon (*Citrus limon*), lime (*C. aurantifolia*) and Rangpur (*C. limonia*) were also analyzed, in which 9.0 ISSR primers generated a total of 84 amplified bands, ranging from 7.0 to 12 with a mean of 9.33 bands per primer, with a 77.4% polymorphism (Zhang *et al.,* 2020). Also, 167 polymorphic bands were produced with an average of 82.74% when 9.0 olive cultivers were tested with 12 ISSR primers, ranging from 11 bands for ISSR18 and 20 bands for ISSR03 (Mohamed *et al.,* 2017). The present study showed high PIC values ranging between (0.878 to 0.979) more than ≧ 0.5, and a high value of gene diversity ranging between (0.883 to 0.980) indicating that the ISSR marker to be highly informative for fig germplasms variance. This is in agreement with Igwe *et al.* (2022) who reported that the PIC value ranged from 0.769 to 0.979 and gene diversity value differed from (0.796 to 0.980). Futhermore, a total of 299 bands were generated. The major allele frequency was 0.142, ranging from 91.21 to 100% polymorphism. When using 9.0 ISSR primers for genetic analysis of 66 accessions of bananas and plantain.

Table 6: ISSR Primers and their Amplification Results Produced in the 66-Fig Germplasms

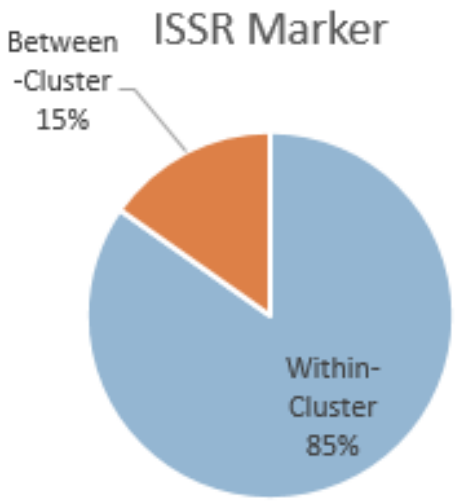
Marker	Primers	MAF	NA	GD	PIC	MI	TAB	TPB	PPB
ISSR	ISSR1	0.152	35	0.944	0.942	9.42	10	10	100
	ISSR11	0.167	43	0.950	0.949	13.28	14	14	100
	ISSR12	0.045	56	0.979	0.979	17.62	18	18	100
	UBC808	0.121	38	0.952	0.950	10.4	11	11	100
	UBC810	0.076	48	0.971	0.970	14.5	15	15	100
	UBC813	0.152	42	0.949	0.947	14.20	15	15	100
	UBC815	0.091	41	0.961	0.960	11.5	12	12	100
	UBC818	0.167	36	0.940	0.938	10.31	11	11	100
	UBC826	0.303	32	0.883	0.878	7.90	9	9	100
	UBC841	0.091	47	0.969	0.968	11.61	12	12	100
	UBC845	0.212	37	0.918	0.914	13.71	15	15	100
	UBC846	0.076	42	0.964	0.963	13.48	14	14	100
	UBC880	0.182	38	0.937	0.934	12.14	13	13	100
	UBC881	0.045	55	0.979	0.978	12.71	13	13	100
	UBC891	0.045	56	0.980	0.979	14.68	15	15	100
	Mean	0.128	43.066	0.952	0.950	12.50	13	13	100

MAF, major allele frequency; NA, number of alleles; GD, gene diversity; PIC, polymorphism information content; MI, marker index; TAB, total amplified bands; TPB, total polymorphic bands; PPB, percentage of polymorphic bands.

The UPGMA dendrogram created among 66 fig accessions by using ISSR market to show genetic variation, or similarity and dissimilarity among them. The relationship among accessions observed with the cophenetic correlation coefficient valued 0.74 between dissimilarity and cophenetic matrices, representing a good fit between two and high accuracy of clustering results (Figure 3). The first cluster (C1) included only one accession (AC01), it was light violet skin color, maroon pulp color, oblate fruit shape and very early maturity period as well as cultivar. The second cluster (C2) comprised of four accessions (AC02, AC03, AC04 and AC07), all of them had yellow skin colors, the first two of them had maroon pulp color and the two others had amber pulp color, all of them had oblate fruit shapes and were cultivar types, with different maturity periods. Furthermore, the third cluster (C3) contained 36 accessions, and divided into 5.0 sub clusters or more then to the most identifications. The first sub cluster consisted of 5.0 accessions (AC08, AC16, AC17, AC18 and AC24). These accessions had different skin colors and two colors of pulp colors maroon and amber, only AC16 had globose fruit shape, the other had an oblate shape. As well as, all of them were cultivars, only AC08 was very late ripened period, the other were mid-season periods. The second sub cluster comprised of 6.0 accessions (AC10, AC14, AC15, AC12, AC11 and AC13). The two accessions AC12 and AC15 were wild types, had dark violet skin color with white and maroon pulp

colors, fruit shape globose and oblate, respectively. The three of AC10, AC13 and AC14 had light red skin colors with oblate fruit shapes, cultivar type as well as two type of pulp colors pink and amber, mid-season and later fruit ripened period. However, other accessions AC11 had yellow skin color, maroon pulp, oblate fruit shape and mid-season ripened period. The third sub cluster included 8.0 accessions (AC59, AC61, AC19, AC20, AC36, AC39, AC37 and AC38). All of them had oblate fruit shapes except AC20 and AC36 which had globose shapes, all of them were mid-season ripened periods and cultivar types except AC37 which was a wild type, violet skin color, yellow pulp, oblate shape, but did not have ripened period because of capri fig type. Most of them yellow skin colors and different pulp colors of pink, amber and red. The fourth sub cluster consisted of 11 accessions (AC33, AC34, AC25, AC26, AC27, AC42, AC28, AC30, AC31, AC29 and AC35). All of them cultivar types, oblate fruit shapes excluding AC27 which was oblong, AC30 globose shape. Most accessions had yellow skin colors except AC34, which was red color, AC26, and AC29 dark violet colors, AC30 green shape. Most of them had midseason ripened periods. The fifth sub cluster had 6.0 fig accessions (AC09, AC21, AC22, AC23, AC05 and AC06). All of them cultivars excluding AC06 which was wild type, light violet fruit skin, and early-ripened period. All accessions had oblate shapes and mid-season ripened periods except AC05 and AC06 with globose shapes, different pulp and skin colors. The fourth cluster (C4) included two (AC32 and AC41), both accessions had dark violet skin colors, yellow pulp, mid-season ripened periods and cultivars, but different fruit shape, AC32 oblate shape, AC41 globose shape. The fifth cluster (C5) had only one accession (AC40). It was yellow skin color, maroon pulp, oblate fruit shape, as well as had mid-season ripened period and of cultivar type. The sixth cluster (C6) consisted of 10 fig accessions divided into two sub clusters, first sub cluster included (AC47, AC48 and AC50), all of them globose fruit shapes, mid-season ripened periods, as well as cultivars. However, AC47 had brown skin color and amber pulp, AC48 light green, amber pulp and AC50 yellow skin color and maroon pulp. The second sub clusters consisted of (AC43, AC44, AC45, AC51, AC52, AC53 and AC54). All of them had oblate fruit shapes, mid-season ripened periods, cultivars excluding AC44, which was wild type and globose shape. AC43, AC44, AC52 and AC54 had violet skin colors and maroon pulp colors except AC44, which had a white pulp color. AC47 had brown skin color and amber pulp color, as well as AC45 and AC50 were yellow skin colored. AC48 and AC53 light green, green skin colors. The seventh cluster (C7) had only one accessions AC46. It was light brown skin color, maroon pulp, oblate fruit shape, mid-season ripened period and cultivar. The eighth cluster (C8) had only one accession AC49. It was brown fruit skin color, amber pulp color, oblate fruit shape, mid-season ripened period and cultivar. The ninth cluster (C9) consisted of two accessions (AC55 and AC56). Both of them oblate fruit shape, mid-season ripened period and cultivar, having two different colors yellow and light green as well as, amber and maroon pulp colors, successively. The tenth (C10) included 8.0 accessions (AC57, AC58, AC60,

AC62, AC63, AC64, AC65 and AC66). All of them had yellow skin colors, excluding AC57 with a green skin color, AC58 brown skin color and AC64 dark violet skin color. As well as, all of them mid-season ripened periods, cultivars and oblate fruit shapes excluding AC63 and AC4, which had globose fruit shapes, with different pulp colors, maroon, amber and pink. Our results are similar or dissimilar with the results of previous studies. Salhi-Hannachi *et al*. (2004) reported that 18 fig varieties based on 4.0 ISSR primers classified into 2.0 major clusters. The UPGMA method classified 23 fig accessions into four major clusters at 0.25 similarity level, when tested with 5.0 ISSR primers (Rout and Aparajita, 2009). The 19 Japanese local fig varieties divided into four clusters by using UPGMA method and detected with a 11 ISSR primers (Ikegami *et al*., 2009). The 21-fig accessions had a dendrogram based on 12 ISSR primers showed two main clusters and a number of sub clusters with a genetic distance ranging between 0.70 and 0.93 (Abdelsalam *et al*., 2019). Based on genetic distances, UPGMA clustering method revealed that 38 Tunisian fig genotypes divided into three main groups, when tested with 6.0 SSR primers (Baraket *et al*., 2011). 76 fig accessions classified into four clusters and numerous sub clusters revealed by Çaliskan *et al*. (2012), when detected with a 10 SSR primers. When tested 90-fig cultivars with a 7.0 SSR primers, clustering UPGMA method showed that fig cultivars classified into three main clusters with three major subgroups (Ganopoulos *et al*., 2015. UPGMA clustering of pairwise genetic distances over 15 SSR loci to represent the genetic relationships among 96 capri fig accessions revealed 7.0 main groups and many subgroups (Çaliskan *et al*., 2018). Ali (2019) distinguished 14-fig genotypes and classified into seven major genetic clusters in the phylogenetic tree, namely C1, C2, C3, C4, C5, C6, and C7, when tested with a 20 SSR primers. A dendrogram clustering method divided the 66 *Musa* accessions into five major groups, based on 9 ISSR primers test, at a similarity index of approximately 0.80 (Igwe *et al*., 2022).



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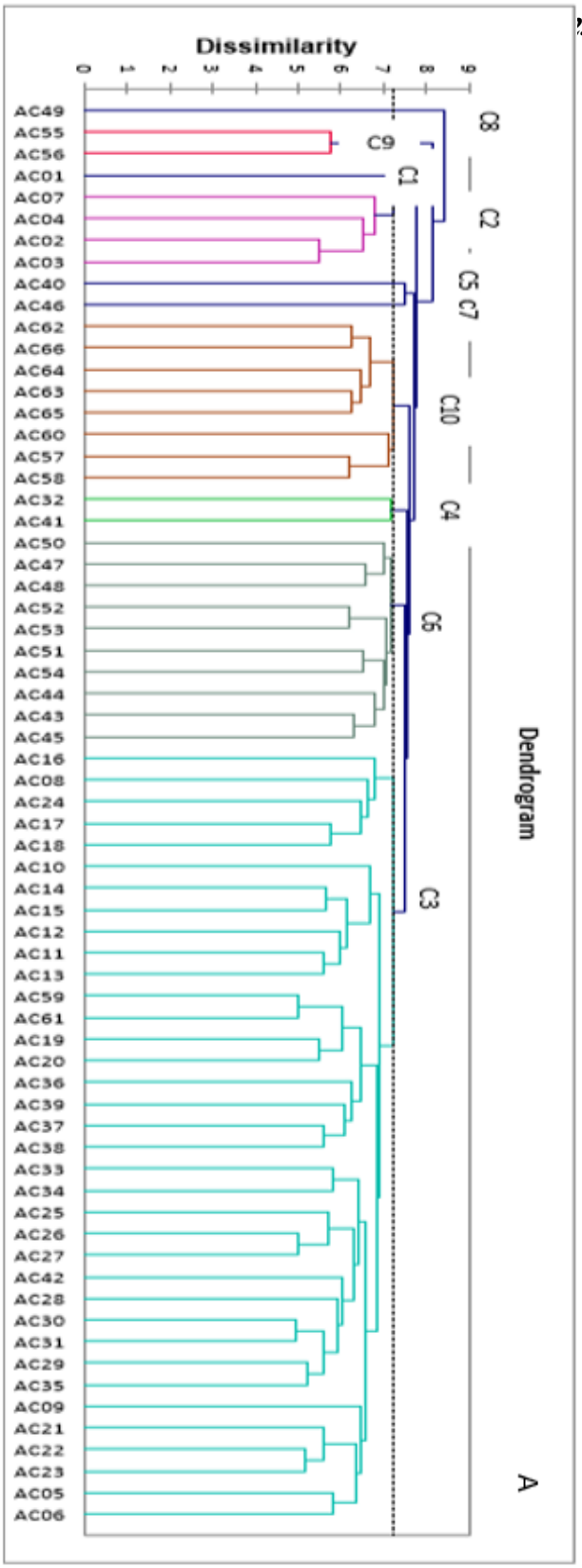

Figure 4. UPGMA dendrongram clusters determined the 66-fig accessions classification.

The STRUCTURE version 2.3.4 software was used to indicate population structure of 66 fig accessions depending on allele frequencies, using of Bayesian-based population approach (Pritchard *et al*., 2000). The results of assessing the 66 fig accessions by 15 ISSR primers were determined to admixture model-based reproductions with K ranging from 1.0 to 9.0. The results demonstrated that the largest delta K value obtained for ISSR marker analysis was (K=2.0) as represented by a sharp peak. Regarding the maximum probability (77%) and according the K value, the 66 fig accessions were classified into 2.0 ideal groups based on ISSR marker (Figure 5). The first population (Red color), included 27 accessions, while 35 accessions were assigned to the second population (Green color), and the remainder 4.0 accessions (AC44, AC3, AC8 and AC2) were considered as the admixed accessions, both accessions (AC44 and AC8), were belonging to the wild types of figs, since they denoted the wild-type gene pool of the other fig cultivars. The results are similar with the results reached by Ganopoulos *et al*. (2015), the maximum for ΔK at (K=2), and structure of 90 fig cultivars classified into 2.0 subpopulations based on 7.0 SSR primers. However, our results are in dissimilarity with Louati *et al*. (2019), who reported that the structure of 66 Argan individuals depending on K value (K=3) and high probability percent, classified into 3.0 sub populations. On the other hand, our results are similar to the results gained by Sheikh *et al*. (2021) who showed that the optimal peak of K was (K=2) thus, structure analysis of 50 apricot accessions classified into 2.0 subpopulations based on 4.0 ISSR primers. The results are also in the affinity with Yilmaz and Ciftci (2021) in that the structure software and delta K mean (K=2) divided 94 laurel genotypes into main populations, with 16 ISSR primers.

Regarding ISSR data between 2.0 populations, inferred clusters value ranged from 0.454 to 0.546. Expected heterozygosity varied from 0.304 to 0.220, with a mean value of (0.262), as well as, the fixation index (Fst) at the range of 0.118 to 0.361 with an average 0.239 (Table 7). The fixation index of population distinction (Fst) assesses diversity on a scale of 0 to 1, with 0.0 representing complete genetic material sharing and 1 representing no sharing. In discriminating populations, an (Fst) values greater than 0.15 can be considered significant (Frankham *et al*., 2002). The fixation index value 0.23 indicating that ISSR marker referred about 23% of fig accessions diversity, this value was highest than the value 0.15 reached by SCoT marker that revealed approximately 15% of diversity among accessions.

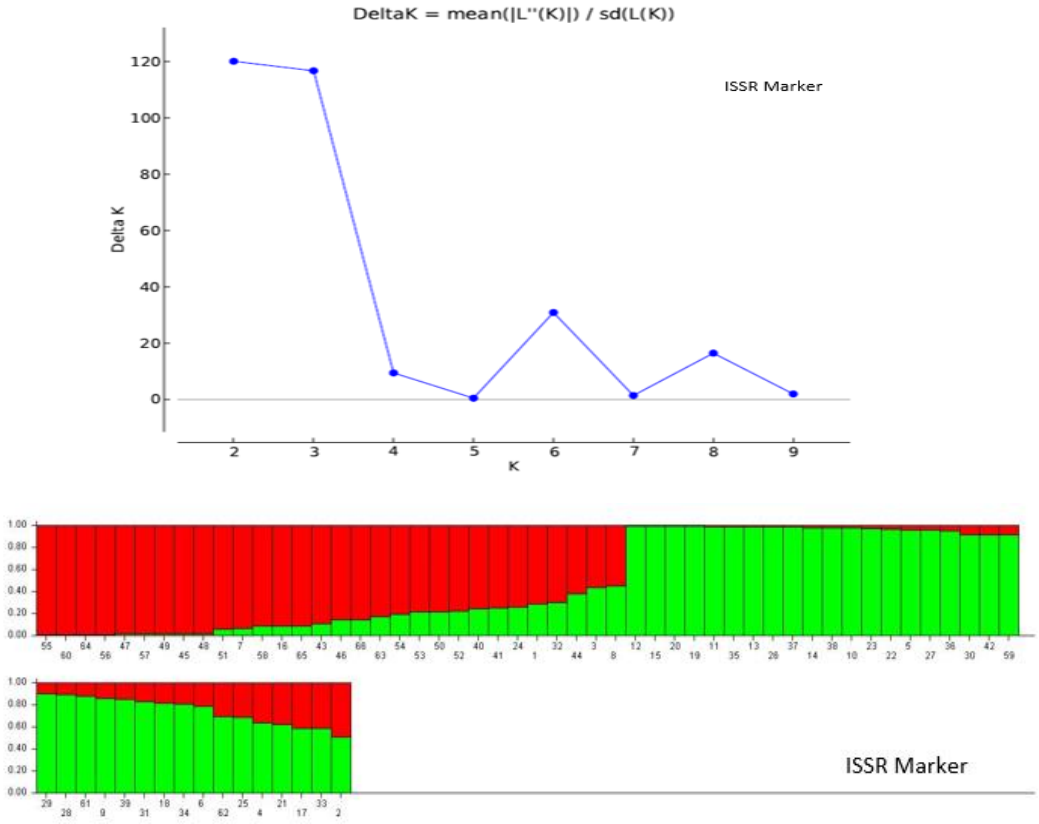

Figure 5. STRUCTURE of 66 accessions based on ISSR data clustered into 2.0 population subsets. Population structure of fig accessions at K = 2.0. Each color represents a specific population subset. The horizontal axis numbers (1–66) correspond to the individual codes of fig accessions.

Table 7: A Structure Illustrating of 66 Fig Accessions, Inferred Clusters, Expected Heterozygosity and Fixation Index Values

Population	Inferred clusters	Expected heterozygosity	Fixation index
Population 1	0.454	0.304	0.118
Population 2	0.546	0.220	0.361
Mean	0.5	0.262	0.239

CONCLUSION

The results illustrated that the morphological and genetic markers are the useful tools to assessment of variability and relationship among fig accessions. Kurdistan region considered as a part of the native of figs (*Ficus carica* L.). Which is a rich source of the fig germplasms, especially wild types. According to morphological traits, fig accessions divided into about five similar groups with a PCA analysis, as well as into five close relationships by JMP Pro 16 software. The results indicated

that the accessions had high ranges of diversity. Genetic diversity analysis is significant not only for crop improvement, but also for efficient management and preservation of the germplasm. The ISSR marker have dominance and co-dominance for identification and estimation of the similarity and variation among the plant germplasms, because they create different groups of results based on their respective characters. Furthermore, all primers recorded 100% polymorphic bands and higher PIC values of 0.950 and higher values of gene diversity 0.952. These are indicators of the powerful of the marker in finger printing and variation test.

CONFLICT OF INTEREST

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

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