Phytochemical analysis and SCoT molecular Marker used to Determine genetic Diversity of many fig Accessions distributed in Various locations of Sulaymaniyah province

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DOI: https://doi.org/10.36077/kjas/2024/v16i4.12074

Received date: 13/5/2023

Accepted date: 1/8/2023

Abstract

Sulaymaniyah province, Kurdistan region, Iraq, is home to a large collection of wild and domesticated fig (*Ficus carica* L.) germplasms. But a lot of these valuable genetic resources have yet to be recognized or categorized by molecular markers. In this study, the identification and classification of 66-fig germplasms were done using phytochemical traits and SCoT (Start Codon Targeted Polymorphism) molecular marker. Total soluble solids (TSS), soluble sugar contents (SSC), total phenolic contents (TPC), total flavonoid contents (TFC), and antioxidant activity by DPPH all showed a large significant variation. There were 232 bands altogether, ranging from 13 bands for the SCoT03, SCoT12, SCoT14, SCoT15, and SCoT33, to 19 bands for the SCoT20. The polymorphic information content (PIC) ranged from 0.737 (SCoT3) to 0.983 in (SCoT21). The major allele frequency values ranged from 0.030 (SCoT21) to 0.500 (SCoT3). The number of alleles varied from 32 in SCoT3 to 63 alleles in SCoT21. The Marker index (MI) values ranged between 9.58 in SCoT3 and 18.67 in SCoT21. As well as, 15 SCoT primers were used to determine genetic diversity. The principal component analysis indicated that the fig accessions divided into 7.0 association groups. The dendrogram created by ward method classified the fig accessions into 5.0 clusters. Phytochemicals and SCoT marker data showed a wide range of diversity, with significant genetic variation across accessions originating from different

populations. The conclusions of this study could aid in the conservation and further use of fig germplasms.

Key wards: Accession, Phytochemical, SCoT, Diversity, DPPH, Cultivar.

Introduction

Fig (*Ficus carica* L.) is a fruit species that belongs to one of the largest genera, with approximately 750 species. It is a deciduous subtropical woody plant, tree, or shrub which has been used for fruit since ancient times, and grown in most Mediterranean-type climates due to its tolerance for high temperatures and insufficient water regimes (22 and 7) .

Fig fruits contain significant amounts of phytochemicals, antioxidants, minerals, vitamins, and dietary fiber. The fruit is used as fresh, dried, concentrate, and paste forms, or as an ingredient in various preparations (37 and 27) . Phenolic compounds are secondary metabolites of plants that are found in a variety of fruits and vegetables. They are regarded as the most important bioactive compounds found in nature (27). Among fruits and vegetables, fig fruits are rich in anthocyanins, flavonol glycosides, and other polyphenols that might contribute to the high antioxidant capacity of its fruits. Fig fruits have an important commercial value because it was known as a health-supporting compound. Polyphenolic content is commonly higher in the peel compared with fruit flesh or pulp. The accumulation of phenolic metabolites in plants takes place under the effect of both environmental conditions and genetic installation (24 and 38) .

The evaluation of a species' genetic diversity profile and genetic structure supports in determining its current condition and risks, and can thus serve as a foundation for developing acceptable scientific management policies and appropriate conservation initiatives. Conserving genetic diversity is critical for increasing population adaptability to changing environments and maintaining a diverse gene makeup for future genetic improvement (1 and 19).

Molecular markers are superior tools for evaluating genetic diversity, allowing breeders to select significant traits that support the improvement of economic plant productivity. It has been demonstrated that molecular marker data are critical for any

breeding program in order to select promising varieties with desirable traits (8). Therefore, the aim of this study is the identification and classification of 66-fig germplasms based on phytochemical traits and SCoT (Start Codon Targeted Polymorphism) molecular marker.

Materials and Methods

This study was carried out during 2020-2021 in the College of Agricultural Engineering Sciences, University of Sulaimani. The identification and categorization of the current wild and domesticated fig accessions based on similarities and differences among them is one of the study's goals. Sixty-six fig accessions' samples were gathered in Sulaymaniyah province, Kurdistan region of Iraq, at diverse sites (Figure 1). To analyze the phytochemical substances, full-repined fruits were taken from each accession, dried in a clean shade place then stored in a fridge freezer. As well as, young healthy leaves were received from each accession, put directly into liquid nitrogen. After that stored inside a freezer to evaluate the molecular variation (Table 1).

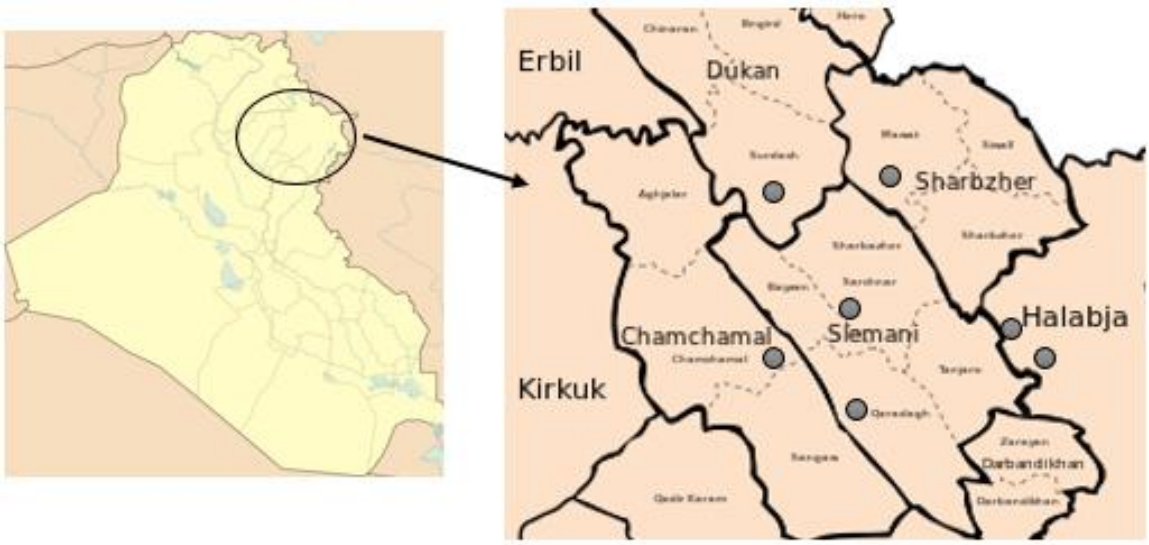


Figure 1. A map shows the location sites of fig accessions distribution.

Table 1. Summarized information about the 66 fig accessions used in this study, which collected in Sulaymaniyah province.

No. of AC	Accessions Code	Location	Accession name	Germplasm types	Skin color

1	AC01	Bazyan	Bazyan-1	Cultivar	Light-Violet
2	AC02	kanipanka	Kanipanka-4	Cultivar	Yellow
3	AC03	kanipanka	Kanipanka-5	Cultivar	Yellow
4	AC04	Dolla Rut	Dolla Rut-7	Cultivar	Yellow
5	AC05	Sharbazher	Sharbazher-12	Cultivar	Yellow
6	AC06	Sharbazher	Sharbazher-13	Wild	Light-Violet
7	AC07	Sharbazher	Sharbazher-14	Cultivar	Yellow
8	AC08	Sharbazher	Sharbazher-15	Wild	Dark-Violet
9	AC09	Sharbazher	Sharbazher-16	Cultivar	Light-Red
10	AC10	Sharbazher	Sharbazher-17	Cultivar	Dark-Red
11	AC11	Sharbazher	Sharbazher-18	Cultivar	Yellow
12	AC12	Sharbazher	Sharbazher-19	Wild-Capri fig	Dark-Violet
13	AC13	Sharbazher	Sharbazher-20	Cultivar	Light-Red
14	AC14	Sharbazher	Sharbazher-21	Cultivar	Light-Red
15	AC15	Sharbazher	Sharbazher-22	Wild	Dark-Violet
16	AC16	Sharbazher	Sharbazher-23	Cultivar	Yellow
17	AC17	Sharbazher	Sharbazher-24	Cultivar	Yellow green
18	AC18	Sharbazher	Sharbazher-25	Cultivar	Yellow green
19	AC19	Bazyan	Bazyan-2	Cultivar	Yellow
20	AC20	Bazyan	Bazyan-3	Cultivar	Yellow green
21	AC21	Dukan	Dukan-27	Cultivar	Bright-Yellow
22	AC22	Dukan	Dukan-28	Cultivar	Yellow
23	AC23	Dukan	Dukan-29	Cultivar	Dark-Violet
24	AC24	Dukan	Dukan-30	Cultivar	Green
25	AC25	Shabazher	Sharbazher-26	Cultivar	Yellow
26	AC26	Kanipanka	Kanipanka-6	Cultivar	Dark-Violet
27	AC27	Khurmall	Khurmall-33	Cultivar	Light-Green
28	AC28	Khurmall	Khurmall-34	Cultivar	Yellow
29	AC29	Khurmall	Khurmall-35	Cultivar	Dark-Violet

30	AC30	Khurmall	Khurmall-36	Cultivar	Green
31	AC31	Khurmall	Khurmall-37	Cultivar	Yellow
32	AC32	Khurmall	Khurmall-38	Cultivar	Dark-Violet
33	AC33	Qaradax	Qaradax-39	Cultivar	Yellow
34	AC34	Qaradax	Qaradax-40	Cultivar	Red
35	AC35	Qaradax	Qaradax-41	Cultivar	Yellow
36	AC36	Qaradax	Qaradax-42	Cultivar	Violet
37	AC37	Qaradax	Qaradax-43	Wild-Capri fig	Dark-Violet
38	AC38	Qaradax	Qaradax-44	Cultivar	Yellow
39	AC39	Qaradax	Qaradax-45	Cultivar	Red
40	AC40	Tasluja	Tasluja-46	Cultivar	Yellow
41	AC41	Tasluja	Tasluja-47	Cultivar	Dark-Violet
42	AC42	Tasluja	Tasluja-48	Cultivar	Yellow
43	AC43	Tasluja	Tasluja-49	Cultivar	Dark-Violet
44	AC44	Byara	Byara-56	Wild-Capri fig	Dark-Violet
45	AC45	Byara	Byara-57	Cultivar	Yellow green
46	AC46	Byara	Byara-58	Cultivar	Light-Brown
47	AC47	Tasluja	Tasluja-50	Cultivar	Brown
48	AC48	Tasluja	Tasluja-51	Cultivar	Light-Green
49	AC49	Tasluja	Tasluja-52	Cultivar	Brown
50	AC50	Tasluja	Tasluja-53	Cultivar	Yellow
51	AC51	Tasluja	Tasluja-54	Cultivar	Brown
52	AC52	Dolla Rut	Dolla Rut-8	Cultivar	Violet
53	AC53	Dolla Rut	Dolla Rut-9	Cultivar	Green
54	AC54	Dolla Rut	Dolla Rut-10	Cultivar	Dark-Violet
55	AC55	Dolla Rut	Dolla Rut-11	Cultivar	Yellow
56	AC56	Dukan	Dukan-31	Cultivar	Light-Green
57	AC57	Dukan	Dukan-32	Cultivar	Green
58	AC58	Taluja	Tasluja-55	Cultivar	Brown

59	AC59	Khurmal	Khurmall-59	Cultivar	Yellow
60	AC60	Khurmal	Khurmall-60	Cultivar	Yellow
61	AC61	Khurmal	Khurmall-61	Cultivar	Yellow
62	AC62	Khurmal	Khurmall-62	Cultivar	Yellow
63	AC63	Khurmal	Khurmall-63	Cultivar	Yellow
64	AC64	Khurmal	Khurmall-64	Cultivar	Dark-Violet
65	AC65	Khurmal	Khurmall-65	Cultivar	Yellow
66	AC66	Khurmal	Khurmall-66	Cultivar	Yellow

Phytochemical tests

Total soluble solids (TSS, ^{o}Birx)

TSS was evaluated using a conventional protocol. The TSS in the juice was determined using a handheld refractometer (ATAGO, Pocket PAL-2, Japan). Three ripened fruits were taken from each accession, the fruits were squeezed until the juice was extracted, and a known volume of juice (drop) was poured on top of the refractometer in the designed spot, after cleaning and calibrating the refractometer. The results were then expressed using the ^{o}Brix unit (9).

Soluble sugar content (SSC)

Soluble sugar content was calculated using the method given by Lateef, Mustafa and Tahir (18), 0.1 g of powdered fruit tissue was taken, added 1000 µL distilled water, and shaken for 20 minutes. The samples were boiled at 92 °C for 30 minutes and cooled by cold water after that centrifuged for 12 minutes at 8000 rpm, the supernatant was taken. To create the standard curve, a series of dilutions (0, 6, 12, 24, 36, 48, 60, 72, 144, 288 and 576 µg.mL^{-1}) were tested. The relationship between the 630 nm absorbance values and the glucose concentrations observed to be linear. Anthrone reagent was prepared by dissolving 0.41 g of anthrone with 44 mL distilled water and then added 231 mL H_2SO_4. 25µL of the supernatant was mixed with 2 mL of anthrone reagent. The solution mixture was incubated at 95 °C for 7 minutes; the color of the solution was changed to dark green. The solutions were cooled, and contrast the blank was read (anthrone reagent solution) at 620 nm and a UV-visible spectrophotometer (UVM6100,

MAANLAB AB, Sweden) was used. Soluble sugar content was calculated by the following formula:

$$SSC\ (\mu g.g^{-1}\ FW) = \frac{V}{W} \times C$$

Where V is the volume of extract (mL), W is the dry weight of fruit sample (g), and C is the concentration of glucose obtained from the standard curve (Table 2).

Total phenolic content

Total phenolic (TP) content of whole fig fruit extracts was determined using the Folin–Ciocalteu as reported by (18 and 39). 0.1 g of powdered fruit tissue was mixed with 1 mL of 60% (v/v) of acidic methanol (Methanol + HCl in a ratio 99:1), and shaken for 40 minutes, then samples incubated overnight at 5 °C. The mixture was centrifuged at 12000 rpm for 15 minutes and the supernatant was collected for TPC analysis. Then 37.5 µL of the supernatant was taken and mixed with 1050 µL of 1:9 Folin–Ciocalteu reagent: water (v/v) after 7.0 minutes as well as, added 850 µL 10% Na_2CO_3 and incubated in dark for 30 minutes. After reaction, the solution color was converted to light blue. The absorbance was measured at 750 nm against the blank (150 µL dH_2O mixed with 1050 µL 1:9 Folin–Ciocalteu reagent: water (v/v) and 850 µL 10% Na_2CO_3). UV-visible spectrophotometer (UVM6100, MAANLAB AB, Sweden), using triplicate time repeated samples. Gallic acid was determined as a standard, and the total phenolic content in each sample was estimated using the standard curve (Table 2). The following equation was used to calculate theTPC:

$$TPC\ (\mu g\ GAE.g^{-1}\ FW) = \frac{V}{W} \times C$$

Where V is the volume of extract (mL), W is the dry weight of the fruit sample (g), and C is the concentration of gallic acid collected from the standard curve.

Total flavonoids content

From the dried fruit powder, 0.1 g was extracted with 1000 µL of 60% (v/v), acidic methanol (methanol HCl in a ratio of 99: 1) and 40% deionized water then, incubated at 10 °C for 16 hrs. The mixture was centrifuged at 14000 rpm at 4 °C for 19 min and the supernatant (extract) was kept and used for total flavonoid content (TFC) estimation according to (18 and 39), the TFC in each extract was calculated. A concentration of 1 $mg.mL^{-1}$ was gained by dissolving 11 mg of quercetin in 11 mL of deionized water to make the stock solution.

Standard curve for quercetin and linear regression between absorption values at 415 nm and quercetin concentrations have been formed (Table 2). Briefly, 900 µL of methanol (80%), 300 µL of aluminum chloride (2% w/v), 80 µL of potassium acetate (1 M), and 1.7 mL of deionized water were combined with 200 µL of juice. At 415 nm, the absorbance of the solution was calculated after 30 min of incubation at 28 °C using a UV-visible spectrophotometer (SHIMADZU, Japan). To accurate accessions, mean values of TFC calculated, each assay was carried out in triplicate. The total flavonoids content of each extract was expressed as the equivalent of µg quercetin (QE) per gram of dry fruit matter by the formula:

$$TFC\ (\mu g\ QE.g^{-1}\ FW) = \frac{V}{W} \times C$$

Where V is the volume of extract (mL), W is the dry weight of the whole fruit (g), and C is the concentration of quercetin determined from the standard (Table 2).

Antioxidant activity by DPPH assay

The antioxidant capacity of the dried fruit juice was determined using the 1-diphenyl-2-picrylhydrazyl (DPPH) radical-scavenging method used by (39). Taken 0.1 g of powdered fruit tissue was added into 1 mL of 60% (v/v) of acidic methanol (99% Methanol+1% HCl) and 40% H_2O, and shaken for 40 minutes. Samples incubated overnight at 5 °C. After that, samples were centrifuged at 12000 rpm for 15 minutes and the supernatant was taken. 37.5 µL of extract was mixed with 1.9 mL of 1-diphenyl-2-picrylhydrazyl (DPPH) solution (0.01g DPPH dissolved in 260 mL of 95% methanol). The mixtures were incubated in dark for 30 minutes at room temperature. To measure samples, the absorbance at 517 nm against the blank (95%methanol) using the UV-visible spectrophotometer (UVM6100, MAANLAB AB, Sweden), each sample repeated three time to accurate the measurements.

The standard compound, 6-hydroxy-2, 5, 7, 8-tetramethylchroman-2-carboxylic acid (Trolox), was used to build the calibration curve. Trolox (12 mg) was combined with 12 mL of 75% ethanol (v/v) solvent and diluted to obtain concentrations of 0.00, 0.33, 0.66, 1.320, 2.00, 2.7, and 3.4 µg/mL. Linear regression was created between the absorbance values at 517 nm and the various Trolox concentrations. The following equation was used:

Antioxidant capacity by DPPH (µg Trolox.g^{-1} FW) = $\frac{V}{W}$ x C

Where V is the volume of extract (mL), W is the dry weight of the fruit sample (g), and C is the concentration of trolox determined from the standard curve (Table 2)

Table 2. Linear equation for standard component of soluble sugar content (SSC), total phenolic content (TFC), total flavonoids content (TFC) and antioxidant activity by DPPH assay.

Component standard	Linear equation	Coefficients of correlation
Soluble sugar content	y= 0.0282x +0.0048	R^2= 0.9952
Total phenolic content	y= 0.0027x-0.0269	R^2= 0.9944
Total flavonoids content	y= 0.0243x + 0.0005	R^2 = 0.9981
Antioxidant activity by DPPH assay	y=0.1368x + 0.0152	R^2= 0.9933

X= represents the mass concentration of the standard curve working solution (µg.g^{-1} FW).

SCoT primers analysis

Fifteen effective SCoT primer designs used by previous studies (2 and 11) were selected based on the highest rate of polymorphisms to evaluate 66 fig accessions in this study .

Table 3. List of SCoT primers, their nucleotide sequences and annealing temperatures (T_m°C).

Name	Sequence (5'-3')	T_m˚C	Name	Sequence (5'-3')	T_m˚C
SCoT2	CAACAATGGCTACCACCC	50.7˚	SCoT15	ACGACATGGCGACCGCGA	59.9˚
SCoT3	CAACAATGGCTACCACCG	51.2˚	SCoT19	ACCATGGCTACCACCGGC	57.1˚
SCoT4	CAACAATGGCTACCACCT	49.5˚	SCoT20	ACCATGGCTACCACCGCG	57.5˚
SCoT10	CAACAATGGCTACCAGCC	51.2˚	SCoT21	ACGACATGGCGACCCACA	56.7˚
SCoT11	AAGCAATGGCTACCACCA	51.4˚	SCoT24	CACCATGGCTACCACCAT	51.6˚
SCoT12	ACGACATGGCGACCAACG	55.9˚	SCoT33	CCATGGCTACCACCGCAG	55.6˚
SCoT13	ACGACATGGCGACCATCG	55.4˚	SCoT35	CATGGCTACCACCGGCCC	57.9
SCoT14	ACGACATGGCGACCACGC	58.6˚			

$T_m{}^oC$; annealing temperatures

Genome DNA extraction

Fresh and healthy leaves from each fig accession (Table 1) were collected and washed by distilled water. The leaf of each accession separately was ground into powder by using liquid nitrogen. An adequate amount of leaf powder (about 300 mg), weighted and transferred to 2.0 mL Eppendorf tube. 1.0 mL of lysis buffer [0.50% (w/v) SDS, 8.00% (w/v) PVP, 0.25 M NaCl, 0.025MEDTA, and 0.2 M Tris-base] and 12 µL RNase (10 $mg.mL^{-1}$) added. After that, tubes were incubated for 70 min at 64 °C and inverted 12 times. The tube samples were cooled at room temperature for 10 min. After cooling, 300 µL of 5M potassium acetate (pH 6.5) was added, mixed, and refrigerated for at least 10 min. The supernatant was also transferred to a new 2 mL tube, centrifuged at 16100 rpm for 15 min. Added 800 µL of chloroform to supernatant, the solution was mixed gently by inversion. The mixture was centrifuged at 14000 rpm for 17 mins, the upper phase was taken and transferred to a new Eppendorf tube (2 mL). 1.0 mL of GE buffer AW1 (2M guanidine thiocyanate diluted in 75% ethanol) was carefully mixed into the upper phase. The mixture was added to a spin column and incubated for 1 min at room temperature. The solution was centrifuged at 8000 rpm for 5 minutes, and the flow solution was discarded. The spin-column was filled with 500 µL of washing buffer AW2 (10 mM NaCl, 10 mMTris-base pH6.5, and 80% ethanol). The flow solution was removed after the column was centrifuged for 5 min at 8000 rpm. A volume of 500 µL of washing buffer AW2 was used to wash the spin column a second time. After centrifuging the spin column at 8000 rpm for 5 min, the flow solution was removed. The spin column was centrifuged for 6 min at 12000 rpm to be dried. The spin column was placed in a new Eppendorf 1.5 mL tube, which was then loaded with 100 mL of elution buffer and incubated at room temperature for 5.0 min. The eluted DNA was collected by centrifuging it at 9000 rpm for 5.0 min and storing it at 20 °C. A NanoDrop spectrophotometer (NanoPLUS, MAALANLAB, Sweden) was used to determine the quantity and purity of purified DNA (1).

PCR amplification

Fifteen repeatable and dependable SCoT primers were selected and used to detect 66

fig accessions. The reaction mixture (25.0 µL) contained 10.0 µL of master mix (AddBio, Korea), 4.0 µL of primer (10 M), 5.0 µL of DNA template (80.0 ng), and 6.0 µL of deionized water (1). Amplification was performed in a Thermo-cycler (Applied Biosystem) for 38 cycles, each of which included a denaturation step at 94 °C for 1 min, an annealing step temperature 50-60 °C for SCoT primers for 1 min, and an extension step at 72 °C for 2 min. A 7-min denaturation step at 94 °C was followed by a 9-min extension step at 72 °C. Amplified products were electrophoresed on a 2.0% agarose gel and stained with Ethidium Bromide 10 µL. DNA fragments was determined using a 1 kb DNA ladder, and the visualized gels images were captured under UV light.

Statistical analysis

One-way ANOVA-CRD with three replications and Duncan multiple range test ($P \leq 0.05$), were used to evaluate significant variations among fig accessions based on phytochemical substances a by using XLSTAT software version 2019. Principal Component Analysis (PCA) and Cluster Analysis by Unweighted Pair-group Method with Arithmetic (UPGMA) dendrogram were used to assess the relationships and differentiations among the accessions. Power Marker version 3.2.5 program was used to calculate major allele frequency (MAF). The polymorphism information content (PIC) was calculated by using the PIC = $1–[f^2 + (1–f)^2]$ formula, where f is the marker frequency in the data set. Marker index (MI) measured by multiplying average of PIC for polymorphic band of each primer (1). Analysis of population structure, a Bayesian model-based analysis was performed using STRUCTURE 2.1 software (30) to identify genetic makeup and showing the number of populations. The admixture model and correlated allele frequencies were used as pedigree and allele frequency models, respectively. The number of probable populations (K) was set between 1 and 9, and the evaluation was repeated three times.

Results and Discussion

Total soluble solids (˚Brix)

The results illustrated high considerable variance among fig accessions, in fruit TSS measurement at the coefficient correlation value (R^2=0.997) (Table 4). The TSS value ranged between (0.00 and 32.00 ˚Brix), the highest value was 32.00 ˚Brix in AC48, it was superior significantly to all the other fig

accessions except AC54, which recorded (31.900 ˚Bx), and both of these are cultivar types. In addition, AC48 has a light green color (greenish), and AC54 had dark violet color with an amber pulp color. However, the lowest value was (0.00 ˚Brix) in (AC12, AC37 and AC44), these accessions were wild (capri fig) types, the syconium of these types have no female flowers, all flowers are male, as well as do not ripen and do not have juice. The results are in affinity with the results found by Özeker and Isfendiyaroglu (26), that the TSS value ranged from 15% to 25.8% among a number of fig varieties. In our study, which have some capri fig types, these types do not hold female flowers and their TSS values considered zero. The highest value of TSS was 15.75% and the lowest was 8.4% found among 22 fig cultivars (21). The highest TSS was 23.53% and the lowest was 18.12% recorded among four fig genotypes (32). Among a number of fig cultivars, TSS value ranged from 13.23 to 19.02 ˚Brix (12). In addition, King *et al.* (17) Showed that the soluble solid concentration ranged between 14.3 and 22.5% among 12 fig cultivars. (33) Reported that the TSS value varied from 20.67 to 23.87% among 6.0 fig genotypes. Marcotuli *et al*. (20) recorded the lowest value 19.1 ˚Brix in 'Petrelli' and the highest value 24.7 ˚Brix in 'Dattato'. The TSS value among forty clones of the Sarilop fig cultivars ranged from 15.8 to 29.8% (35).

Soluble sugar content (SSC)

The results showed high significant variances among fig accessions in soluble sugar contents of dried fruits (Table 4). The highest value of SSC was (110.53 mg.100 g^{-1} FW) found in AC42, followed by (105.34, 100.30, 1001.40 and 97.97 mg. 100 g^{-1} FW), in AC28, AC41, AC48 and AC29, respectively. While, the lowest value was (3.37 mg. 100 g^{-1} FW) found in AC12, followed by 3.63 and 9.27 mg. 100 g^{-1} FW in AC37 and AC44 (both of them are wild-capri fig types). The overall average was 68.25mg.100 g^{-1} FW. These fig trees hold only male flowers, and the fruits do not ripen. The results are similar or dissimilar with the results in the previous studies. Melgarejo *et al.* (23) reported that the sugar concentration in juice of second crops of fig was ranged from 16.17% to 20.35%. Our results are similar with the findings of Hssaini *et al*. (15) referring that the soluble sugar content in fig fruits was varied from 9.77 to 17.71.9 g/100 g^{-1} FW, with an average of 12.08±1.26 g/100 g^{-1} FW. In

addition, Petkova, Ivanov and Denev (29) revealed that the total carbohydrates in fresh figs was recorded 23.5 g.100 g^{-1} FW, in frozen fruit 19.2 g.100 g^{-1} FW and reached 65.9 g.100 g^{-1} FW in fig jam.

Total phenolic content (TPC)

A considerable variance was recorded among fig accessions in total phenolic contents of dried fig fruits (Table 4). The TPC in dry fig fruit ranged from 14.116 mg GAE.100 g^{-1} FW in AC30 to 53.829 mg GAE.100 g^{-1} FW in AC04, which are yellow skin colored. Followed by the high value (45.839, 42.868 and 43.967 mg GAE.100 g^{-1} FW) in AC07, AC13 and AC32, successively, the first accession has a yellow skin color, the second has a light red and the last accession has a dark violet skin color. However, the lowest values of TPC (15.577, 16.288, 16.738 and 16.963 mg GAE.100 g^{-1} FW) were found in AC53, AC27, AC56 and AC64, respectively, with a mean value of 26.054 mg GAE.100 g^{-1} FW. Our results nearly or slightly more than that reached by Vallejo, Marín and Tomás-Barberán (36) who stated that the total phenolic compound content of the dried fig (Cuello Dama) variety was 19.2 mg.100 g^{-1}. As well as showed that skin was the most important donor to the total phenolic content, ranging from 19.1 mg.100 g^{-1} in (Nazaret) to 140.2 mg.100 g^{-1} in (VB1). The pulp gave a much lower phenolic compound content than skin, ranging from the lowest cultivar 0.0 mg.100 g^{-1} FW (Nazaret) to 11.3 mg/100 g^{-1} FW in (CDN9). The same researchers revealed that about 15% of total phenolics were lost in the drying processes, dropping to 19.1 mg.100 g^{-1} FW, while the (Cuello Dama) fresh cultivar recorded 21.8 mg.100 g^{-1} FW. Bey and Louaileche (5) reported that the total phenol content in fig fruit was more than that of our results ranging from 482.62 to 644.11mg.100 g^{-1} FW. Furthermore, they referred that the TPC of the darker varieties was higher than that of the light ones, with an average of 618.85 mg.100 g^{-1} and 514.72 mg.100 g^{-1} FW, respectively. The total phenol content in black fig was differed from 50.57 to 74.16 mg GAE g^{-1} FW. As well as in purple fig, it was ranged from 61.47 to 63.11 mg GAE.100 g^{-1} FW that were more than our results. The total phenolic content (TPC) in black fig, peel and juice recorded high TPCs, followed by total fruit and pulp (14). Our results are in a slight affinity with the results found by Pereira, Serradilla, *et al.* (28) who reported that TPC in both skin and flesh of Cuello Dama Negro

variety was 169.5 and 34.3 mg GAE.100 g^{-1} FW, respectively. However, the lowest value of TPC was 58.9 mg GAE.100 g^{-1} FW and 23.3 mg GAE.100 g^{-1} FW in both skin and flesh of Cuello Dama Blanco variety, successively. Hssaini *et al*. (15) Showed that the total phenol content values in fig accessions were in a range of 22 to 417.56 mg GAE.100 g^{-1} FW, with a mean of 142.74 mg GAE.100 g^{-1} FW. The highest content of polyphenols was observed in fresh figs, followed by fig jam and it was the lowest in frozen fruit 31.0, 28.5 and 14 mg GAE.100 g^{-1} FW, respectively (29). In addition, they referred that the freezing causes a decrease in phenol contents of fig fruits.

Total Flavonoids content (TFC)

The total flavonoid concentrations of fig fruits differed significantly among different fig accessions (Table 4). The highest value of TFC was 24.191 mg QE.100 g^{-1} FW in AC43, followed by (20.558, 19.811 and 18.386 mg QE.100 g^{-1} FW) in AC13, AC32 and AC04, respectively. However, the lowest value of TFC was (5.577 mg QE.100 g^{-1} FW) in AC30, followed by the values (5.876, 7.300 and 7.400 mg QE.100 g^{-1} FW) in AC58, AC55 and AC44, respectively with a mean of 13.226 mg QE.100 g^{-1} FW. The results agree and disagree with the results of previous studies. Bey and Louaileche (5) reported that the total flavonoids content in dark varieties (Bouankik, Azandjar and Aberkane) were more than light ones, with mean values of 126.55 and 87.24mg.100 g^{-1} FW, respectively, which were higher than those found in our results. The highest value of flavonoids concentration was recorded in leaf (95.62±5.2 mg catechin.100 g^{-1} FW), meanwhile, the least content was observed in pulp as 4.49±0.25 mg catechin.100 g^{-1} FW (3). Our results are in affinity or a bit more than that of Harzallah *et al.* (14) who revealed that the total flavonoid content (TFC) in the peel of Kohli fig variety recorded the highest concentration of flavonoids (12.75 mg CE.g^{-1} FW), while, in total fruit of Hamri variety, it was (11.30 mg CE.g^{-1} FW). Total flavonoids (TF) in dried fig fruit ranged from 8.11 to 112, with an average of 42.04 mg CE.100 g^{-1} DW (15). In addition, our results are similar with the results found by Petkova, Ivanov and Denev (29) who reported that the total flavonoids in fresh figs were the highest (11.4 mg QE.100 g^{-1} FW) followed by fig jam (6.6 mg QE.100 g^{-1} FW), while, in frozen figs, it was the lowest (3.6 mg QE.100 g^{-1} FW). The fruit

development stage, fruit drying and freezing reduce the total phenols and flavonoid contents in fruits (14 and 29).

Antioxidant activity by DPPH assay

High significant variations found among fig accessions in antioxidant activity by detection of DPPH in the juice of dried fig fruits (Table 4). The highest antioxidant activity by DPPH assay was 118.779 mg Trolox eq.100 g^{-1} FW in AC41, which is a dark violet skin color and cultivar type. Subsequently (116.932, 116.392 and 115.941 mg Trolox eq.100 g^{-} F.W.) were recorded in AC55, AC44 and AC60, successively. AC44 is of a dark violet skin color, white pulp and wild type. However, the lowest value was 35.716 mg Trolox eq.100 g^{-1} FW in AC64, followed by (61.930, 69.545 and 70.131 mg Trolox eq.100 g^{-1} FW) in AC43, AC45 and AC31, respectively, with a mean of 82.796 mg Trolox eq.100 g^{-1} FW. Our results are similar and dissimilar with the results reached by previous researchers. The antioxidant activity, DPPH assay values was observed among fig leaf, peel and pulp samples in the order of 59.55±4.6, 49.44±3.8 and 27.33±2.2%, respectively (3). The antioxidant activity was measured in all three parts (peel, pulp, total fruit) juices of three fig varieties within a concentration range of 2.0 to 20 $mg.mL^{-1}$ for DPPH assay (14). Radical scavenging activity, expressed as EC_{50}, ranged from 1.20 to 1.49 $mg.g^{-1}$ FW, thus Bursa Siyahi fig cultivar has the smallest EC_{50} value (1.20±0.028 $mg.g^{-1}$ FW) value and showed the highest antioxidant activity (10). Total antioxidant activity (TAA) of both skin and flesh of figs, in dark-colored varieties, such as 'Colar Elche' and 'Cuello Dama Negro' were 177.4 and 109.4 mmol Trolox eq.100 g^{-1} FW, respectively (28). Furthermore, the antioxidant activity, DPPH assay ranged from 34.61 to 1468.41with an average of 541.88±53.3 mM Trolox eq.100 g^{-1} DW (15). The highest antioxidant activity was accounted in fresh figs (21.3±1.2 mM TE.100 g^{-1} FW for DPPH assay (29). The phytochemical compounds of fig comprising soluble sugar content, total phenols, total flavonoids, antioxidant activity and total soluble solids influenced by the ripening stage, environment conditions (land topography, temperature and light direction), agronomic services as well as, varieties of fig.

Total flavonoids / Total phenols percent

The fig accessions showed considerable differences in the proportion of total flavonoids in total phenols in whole parts of fruit (Table 4). The TFC/TPC ratio was ranged from 24.5% in (AC44) to 74.1% in (AC66); the first accession is of a dark violet and wild type and the second is of a yellow skin type. As well as, AC06, AC01 and AC65 recorded the higher ratios (72.1, 70.8 and 70.5%), respectively; all of them are light types. However, AC51, AC58 and AC04 had lower values (27.2, 29.2 and 34.1%), successively. These results indicate that most of the total phenols in light fruit skinned colors are flavonoids, as well as referred that flavonoid is a part of phenols. The results nearly agree with that reached by Hssaini *et al*. (15) who revealed that the ratio between total flavonoids to total phenols was in the range of 0.09 to 0.87 among a number of fig accessions.

Table 4. Measurement of soluble sugar contents, total phenol contents, flavonoid contents, antioxidant activity, total flavonoids/total phenols ratio and total soluble solid in the juice of different parts of fig fruits.

Accessions	Soluble sugar content (SSC, mg Glu.100 g^{-1} dFW)	Total phenol content (TPC, mg GAE.100 g^{-1} dFW)	Total flavonoids content (TFC, mg QE.100 g^{-1} dFW)	Antioxidant activity (DPPH assay, mg Trolox eq.100 g^{-} dFW)	Total flavonoids/ Total phenolic	Total soluble solids (^{o}Brix)
AC01	60.963 yz	18.336 aa-o	12.993 v	79.230 st	70.8	18.367p-s
AC02	86.951 i	27.787 r	14.678 o	74.275 a-ad	52.8	20.433o
AC03	59.564 z-aa	24.453 x	14.740 no	78.419 tuv	60.2	18.433pqr
AC04	95.346 ef	53.829 a	18.386 d	75.851 xy	34.1	17.200tuv
AC05	51.107 aa-g	23.829 aa	13.404 t	78.464 tuv	56.2	25.367g
AC06	28.700 aa-k	19.210 aa-l	13.854 s	88.824 k	0.721	30.607b
AC07	76.457 pq	45.839 b	18.261 e	70.176 a-ah	39.8	25.033gh
AC08	68.350 v	24.041 yz	11.607 a-ab	82.745 nop	48.2	25.700fg
AC09	69.399 uv	27.787 r	15.764 k	73.284 a-ae	56.7	18.733pq
AC10	63.206 wx	28.835 p	16.114 j	80.356 qr	55.8	18.767p

AC11	84..67 kl	23.567 aa-b	12.693 x	73.554 a-ae	53.8	20.267o
AC12	3.370 aa-n	21.719 aa-g	13.404 t	83.959 m	61.7	0.000aa
AC13	41.601 aa-j	42.868 d	20.558 b	83.194 mno	47.9	20.400o
AC14	95.372 ef	29.753 o	16.737 i	101.393 g	56.2	17.900rst
AC15	46.992 aa-h	23.991 y-aa	9.809 a-ag	92.203 i	40.8	21.267lm
AC16	51.303 aa-e	39.697 e	17.100 h	78.059 uvw	43	23.567j
AC17	64..32 w	22.556 aa-f	14.416 p	73.869 a-ad	63.9	21.967kl
AC18	96.045 e	23.330 aa-c	12.169 aa	78.779 tu	52.1	26.367ef
AC19	54.996 aa-c	17.487 aa-p	8.273 a-aj	75.041 y-aa	47.3	24.467hi
AC20	72.712 t	20.521 aa-i	10.970 a-ae	94.995 h	53.4	17.967q-t
AC21	56.601 aa-b	17.300 aa-p	8.236 a-aj	70.131 a-ah	47.6	20.433o
AC22	89.008 h	24.453 x	12.655 xy	73.419 a-ae	51.7	19.933o
AC23	91.621 g	25.052 w	12.993 v	72.743 a-af	51.8	21.300lm
AC24	53.844 aa-d	25.652 v	12.993 v	69.545 a-ah	50.6	21.233lmn
AC25	51.774 aa-f	24.828 w	16.740 i	71.680 a-ag	67.4	20.355o
AC26	86.395 ij	31.794 l	13.467 t	91.482 i	42.3	17.833rts
AC27	80.243 mn	16.288 aa-s	9.360 a-ai	74.230 a-ad	57.4	17.533tu
AC28	105.346 b	26.838 s	18.386 d	72.293 a - ag	68.5	16.700v
AC29	97.979 d	36.963 g	14.303 q	97.979 d	38.6	27.300cd
AC30	85.284 jk	14.116 aa-u	5.577 a-ao	74.320 a-ac	39.5	15.567w
AC31	60.798 yz	28.298 q	17.637 fg	79.995 qrs	62.3	17.733rst
AC32	25.619 aa-l	43.967 c	19.811 c	102.879 f	45	16.505v
AC33	58.391 aa	23.167 a-ac	14.066 r	82.743 nop	60.7	14.767x
AC34	75.202 qr	28.985 p	11.270 a-ad	89.905 j	38.8	17.700rst
AC35	64.132 w	25.914 u	13.142 u	78.689 tu	50.7	26.900cde
AC36	62.835 wx	19.060 a-al	12.805 w	80.941 q	67.1	22.400k

AC37	3.638 aa-n	29.522 o	13.067 uv	83.464 mno	44.2	0.000aa
AC38	45.016 aa-i	26.551 t	10.970 a-ae	80.581 qr	41.3	16.833uv
AC39	50.058 aa-g	30.895 m	17.662 f	87.788 l	57.1	27.433c
AC40	53.658 aa-d	23.105 a-ac	14.865m	74.770 a-ab	64.3	23.667j
AC41	100.305 b	23.317 a-ac	12.730 wx	118.779 a	0.545	26.633de
AC42	110.531 a	37.187 f	13.105 u	109.500 e	35.2	26.600de
AC43	61.930 xy	36.625 h	24.191 a	61.930 xy	66	21.633kl
AC44	9.276 am	30.084 n	7.400 a-al	116.392 bc	24.5	0.000aa
AC45	73.535 st	33.067 j	17.562 g	73.535 st	53.1	20.433o
AC46	97.918 d	18.798 a-am	12.531 z	112.338 d	66.6	23.600j
AC47	81.025 m	18.598 a-an	9.434 a-ai	82.113 p	50.7	20.367o
AC48	100.140 c	22.843 a-ae	10.333 a-af	75.176 y-aa	45.2	32.000a
AC49	61.992 xy	22.880 a-ad	9.434 a-ai	75.446 xyz	41.2	12.933y
AC50	62.321 xy	32.206 k	15.390 l	76.347 x	47.7	20.500no
AC51	78.864 no	34.303 i	9.360 a-aj	76.392 x	27.2	21.300lm
AC52	70.243 u	22.655 a-af	10.408 a –af	80.401 qr	45.9	17.567stu
AC53	74.703 op	15.577 a-at	8.124 a-ak	73.779 a-ad	52.1	30.333b
AC54	68.556 v	28.386 q	16.663 i	79.185 st	58.7	31.900a
AC55	76.539 pq	18.422 a-ao	7.300 a-am	116.932 b	39.6	15.500wx
AC56	72.794 t	16.738 a-ar	9.622 a-ah	113.149 d	57.4	15.433wx
AC57	94.132 f	24.154 y	9.884 a-ag	77.923 uvw	40.9	11.633z
AC58	83.144 l	20.109 a-aj	5.876 a-an	77.563 vw	29.2	21.700kl
AC59	75.619 pq	32.955 j	14.790 mn	76.519 pq	44.8	23.833ij
AC60	41.045 a-aj	20.221 a-aj	12.581 yz	115.941 c	62.2	22.200k
AC61	84.996 jk	23.779 aa	12.805 w	73.959 a- ad	53.8	20.400o
AC62	61.025 yz	21.270 a-ah	13.067 uv	77.383 w	61.4	23.567j

AC63	83.267 l	19.884 a-ak	11.382 a-ac	77.833 uvw	57.2	20.400o
AC64	77.403 op	16.963 a-aq	9.809 a-ag	35.716 a-ai	57.8	18.700pq
AC65	72.671 t	25.951 u	18.311 de	79.725 rs	70.5	20.333o
AC66	68.144 v	21.632 a-ag	16.039 j	75.941 xy	74.1	24.167ij
Mean	68.254	26.054	13.226	82.796	52	20.28

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

Multivariate analyses among fig accessions based on phytochemical substances

The principal component analysis (PCA) is used to discriminate the most significant descriptors in the data set. As a result, the five principal components (factors) determined 100% of variances among the fig accessions of five phytochemical characters (Table 5). The first two factors had the eigenvalue more than one (>1.0) which described 63% of variances among the accessions for phytochemical traits. The first factor gave 33.841% of total variance, and had a strong positive correlation with TFC (r=0.920**) and TPC (r=0.874**), while it had a weak negative correlation with total antioxidant activity (r=-0.174). The second factor revealed the 29.369% of total variances, which have strong positive correlations with SSC (r= 0.821**) and TSS (r= 0.811**), however, weak negative correlations with TPC and TFC (r= -0.189) and (r= -0.089), respectively, and a strong negative correlation with DPPH (-0.304*). The third factor determined 19.587% of total variances, had a strong positive correlation with DPPH only (r= 0.926**) and a weak negative correlation with TFC (r=-0.044). Furthermore, the bi plot axes clarified that the fig accession scattered onto four quarters. As well as, the accessions composed of about seven closely related groups (Figure 2). The most of accessions collected in GrII, then, GrI. In addition, the third group (GrIII) consisted of 4.0 fig accessions (AC16, AC31, AC39, and AC45), three of them were yellow and yellow green colors except AC39 which had a red skin color. The fourth group (GrIV) included 3.0 accessions (AC04, AC07 and AC43), the first two accessions were yellow and the last accession was a dark violet. The fifth group (GrV) had 2.0 accessions (AC13 and AC32) with light red and dark violet colors, respectively. The sixth group (GrVI) comprised of 3.0 wild type accessions

(AC12, AC37 and AC44), all of them were dark violet skin colors and capri fig types. The last group (GrVII) included 2.0 accessions (AC55 and AC56), with yellow and light green fruit skin colors. Both descriptors SSC and TSS had strong correlations with each other, as well as, both of them had effects on the second factor with a medium effect on the variation compared with TFC and TPC which had the highest effect on the variations, as well as, a strong correlation existed between them. The descriptor DPPH had a weak effect on the variation and negatively correlated with the (SSC and TSS) and (TFC and TPC).

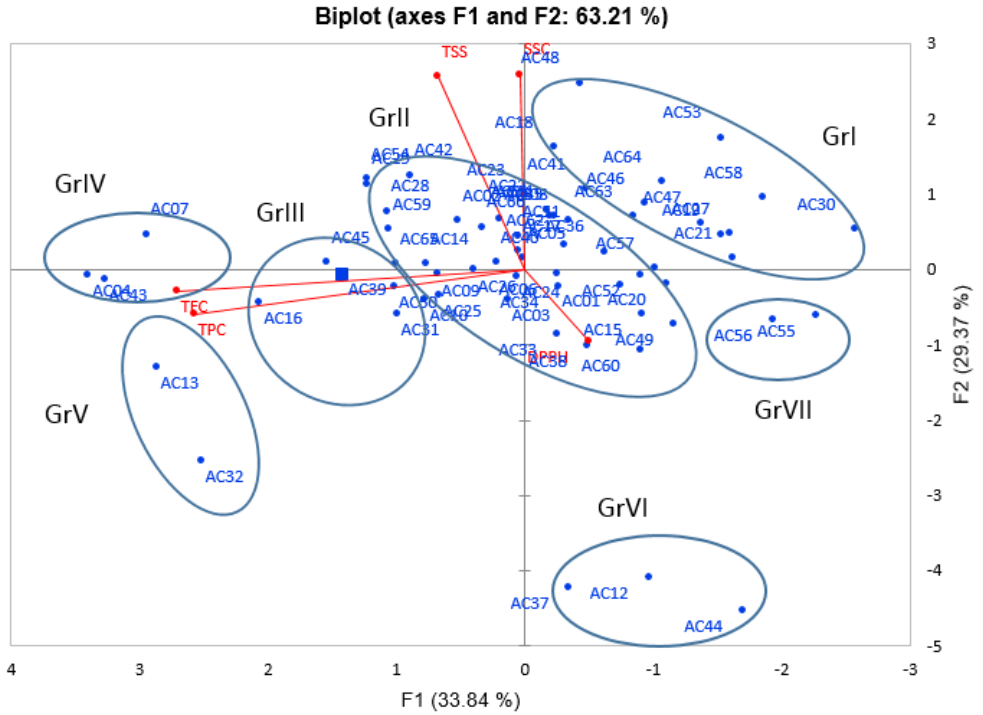


Figure 2. Principal component analysis, bi plot demonstration of correlation, variances, and accessions grouping based on phytochemical variables.

Table 5. Determinate of the correlation between variables and factors, eigenvalue, percent variability and a percent cumulative variability among factors and variables.

Variables	F1	F2	F3	F4	F5
Soluble sugar content	0.012	0.821	0.260	-0.493	0.125
Total phenols content	0.874	-0.189	0.192	-0.236	-0.328

Total flavonoids content	0.920	-0.089	-0.044	0.128	0.357
Antioxidant activity, DPPH assay	-0.172	-0.304	0.926	0.126	0.068
Total soluble solid	0.229	0.811	0.124	0.505	-0.139
Eigenvalue	1.692	1.468	0.979	0.585	0.275
Variability (%)	33.841	29.369	19.587	11.704	5.499
Cumulative %	33.841	63.211	82.797	94.501	100.000

SSC: soluble sugar content, TPC: total phenol content, TFC: total flavonoids content, AA (DPPH): antioxidant activity, TSS: total soluble solids.

Assessment of genetic diversity among fig accessions using molecular marker (SCoT)

Based on the competence of polymorphic bands generation, fifteen SCoT primers were selected in the previous studies (2, 11, 16 and 31).

The fifteen SCoT primers, which were determined to test the genetic diversity among 66 fig accessions, are shown in (Table 6). A total of 232 polymorphic bands generated with a 100% polymorphism. The highest number of bands (19) was observed in (SCoT20 and SCoT21), followed by 18 bands in (SCoT11 and SCoT19), and 16 bands in (SCoT4 and SCoT10), while the lowest number of bands (13) was recorded from (SCoT3, SCoT12, SCoT14, SCoT15 and SCoT35), then 14 bands in SCoT2, with an average of 15 bands. The major allele frequency values ranged from 0.030 in (SCoT21) to 0.500 (SCoT3), followed by 0.318, 0.242, 0.227 and 0.197 in (SCoT2, SCoT33, SCoT10 and SCoT13), respectively, with a mean of 0.160. As well as the number of alleles varied from 32 in (SCoT3) to 63 alleles in (SCoT21), followed by 58 alleles in (SCoT4), 55 alleles in (SCoT11 and SCoT20) and 45 alleles in (SCoT15), with a mean 48.133 alleles. The highest gene diversity value 0.983 found in (SCoT21) followed by 0.77 in (SCoT13), 0.975 in (SCoT15) and 0.974 in (SCoT4 and SCoT19), while the lowest value 0.742 was found in (SCoT3), followed by 0.878, 0.923, 0.927 in (SCoT2, SCoT10 and SCoT33), successively, with a mean 0.942. Furthermore, the highest value of

polymorphism information content (PIC) was 0.983 recorded by SCoT21, followed by 0.976, 0.975 and 0.974 in (SCoT13, SCoT15 and SCoT4), respectively. However, the lowest value 0.737 was noticed in (SCoT3), then 0.878, 0.923 and 0.925 in (SCoT2, SCoT10 and SCoT33), successively with an average of 0.940. The Marker index value ranged between 9.58 in (SCoT3) and 18.67 in (SCoT21). As well as, SCoT20, SCoT19 and SCoT11 gave the high marker index values 18.39, 17.51 and 17.44, respectively. While, SCoT2, SCoT14 and SCoT12 gave lower marker index values 12.23, 12.32 and 12.5, respectively with a mean of 14.59. Our results are so close to the results obtained by Ismail *et al*. (4) in the number of bands, it was ranged from 6.0 (SCoT-11) and 19 (SCoT-03), with a mean of 13.5 bands of the 135 bands as a total. However, differ in polymorphism percent, with the range of 0.0 in (SCoT-11 and SCoT-22) to 24% in (SCoT-16) when tested 10 fig sample plantlets with 10 SCoT primers, which is the only study on fig trees using SCoT primers. Our results are so near to the results found by Mohamed *et al*. (25) who studied 9.0 olive varieties, when examined with 13 SCoT primers. A total of 242 bands produced, with a mean of 18.6 bands per primer. The number of polymorphic bands ranged from 12 bands to 31 bands. SCoT2, SCoT11, and SCoT 22 showed percentages of 94.7, 88.9, and 94.1%, respectively, 10 primers showed 100% polymorphism. Furthermore, 201 polymorphic bands were generated, when investigated 50 varieties of (*Bletilla striata*), with 20 SCoT primers. The number of polymorphic bands ranged from 6.0 to 13, with a 96.17% polymorphism. The PIC value ranged from 0.92 to 0.99, with a mean of 0.96 (13). A total 61 polymorphic band (83% polymorphism) were produced when tested 48 safflower accessions by 10 primers of SCoT marker. The highest and lowest number of polymorphic bands was 11 bands in (SCoT35), while the lowest 3.0 bands in (SCoT22). The PIC values for 10 primers differed from 0.22 (SCoT 1) to 0.48 (SCoT 35) with an average of 0.39 per primer. The marker index (MI) of the primers differed from 0.88 (SCoT 1) to 5.28 (SCoT 35) (34). Our results more than the results reported by Ahmed *et al.* (2) in which a total of 93 bands were produced, out of which 54 bands with 58% polymorphism, across 82 barley genotypes detected with 10 SCoT primers. The number of polymorphic bands were ranged from 4.0 to 7.0 bands. PIC values varied from 0.23 (SCoT11) to

0.43 (SCoT28) with an average of 0.33 per primer, Marker index (MI) of the primers varied from 1.04 (SCoT12) to 3.01 (SCoT28). When ninety-four laurel genotypes were tested with 10 SCoT primers, 227 bands as a total and 175 of them polymorphic bands with a (77.1%) polymorphism. The number of polymorphic bands were ranged from 5.0 to 28 bands. The PIC values was varied between 0.15 in (SCoT08) and 0.63 in (SCoT05), with an average PIC was 0.45 (40). The results are confirmed by Igwe *et al*. (16), when they detected accessions of bananas and plantains from different genomes with 8.0 SCoT primers. A total of 326 numbers of alleles were generated. The number of polymorphic loci ranged from 64 to 66. The percent of polymorphism ranged from 96.97 to 100%. The major allele frequency was ranged from 0.0455 to 0.3736, with a mean of 0.1717. The gene diversities varied from 0.832 to 0.9829, with a mean of 0.9432. The PIC value varied from 0.8214 to 0.9986, with an average of 0.9421. SCoT markers showed a high average value of PIC 0.940 more than ≧ 0.5, and a high average value of gene diversity 0.942, as well as, high average value of allele numbers 48.133. These results indicated that the marker is highly informative for detecting fig germplasms variability.

Table 6. Efficacy and polymorphism of 15 SCoT sequence primers employed in *F. carica* accessions.

Marker	Primers	MAF	NA	GD	PIC	MI	TAB	TPB	PPB
SCoT	SCoT2	0.318	34	0.878	0.874	12.23	14	14	100
	SCoT3	0.500	32	0.742	0.737	9.58	13	13	100
	SCoT4	0.106	58	0.974	0.974	15.58	16	16	100
	SCoT10	0.227	41	0.926	0.923	14.76	16	16	100
	SCoT11	0.121	55	0.970	0.969	17.44	18	18	100
	SCoT12	0.197	39	0.938	0.935	12.15	13	13	100
	SCoT13	0.061	53	0.977	0.976	14.64	15	15	100
	SCoT14	0.152	41	0.950	0.948	12.32	13	13	100
	SCoT15	0.076	54	0.975	0.975	12.67	13	13	100
	SCoT19	0.061	50	0.974	0.973	17.51	18	18	100
	SCoT20	0.121	55	0.969	0.968	18.39	19	19	100

	SCoT21	0.030	63	0.983	0.983	18.67	19	19	100
	SCoT24	0.106	50	0.967	0.967	16.43	17	17	100
	SCoT33	0.242	46	0.927	0.925	13.87	15	15	100
	SCoT35	0.076	51	0.973	0.973	12.64	13	13	100
	Mean	0.160	48.133	0.942	0.940	14.59	15	15	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.

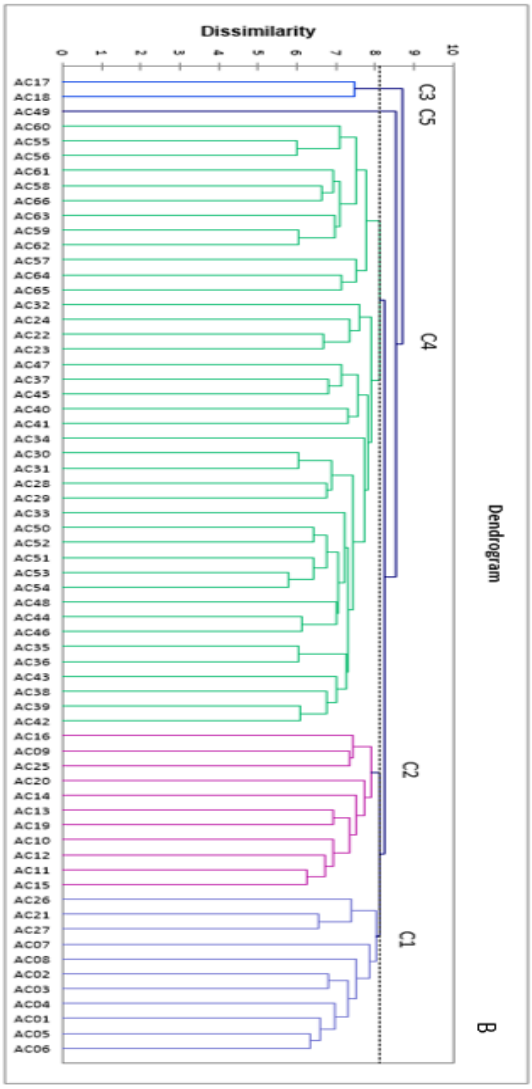
Cluster analyses among fig accessions based on SCoT marker

Fig accessions clustering with UPGMA clustering method based on SCoT marker was used to determine the similarity and dissimilarity among them and created with UPGMA dendrogram. The relationship among accessions demonstrated with the cophenetic correlation coefficient value 0.62 between dissimilarity and cophenetic matrices, demonstrating a good fit between two and high correctness of clustering results (Figure 3). The fig accessions classified into 5.0 main clusters, and numerous sub clusters with different distances among clusters. The lowest value of distance (d=3.44) was noticed between C1 and C4, followed by (d=3.52) between C2 and C4 and (d= 3.65) between C1 and C2. While, the highest value of distance was (d=8.14) found between C3 and C5, then (d=7.62) between C2 and C5, and (d=7.08) between C1 and C5) (Table 7). The percent of variance 92% of total variance recorded within the clusters as well as, 8% of total variance between the clusters were observed (Figure 4). The first cluster (C1) included 11 fig accessions and divided into two subclusters, the first subcluster consisted of 3.0 accessions (AC26, AC21 and AC27), all of the accession are cultivars, with different skin colors; dark violet, yellow and light green, different pulp colors; amber, red and pink, respectively. While AC26 and AC21 had oblate fruits, except AC27 with an oblong fruit shape, the second subcluster comprised of 8.0 accessions (AC01, AC02, AC03, AC04, AC05, AC06, AC07 and AC08), all of them had yellow skin colors excluding AC01 and AC06, which had a light violet color. All accessions had oblate fruit shapes except AC05 and AC06 with globose shapes, as well as, are cultivars except AC06 which is a wild type, with

different pulp colors; maroon, red and amber and different ripening periods. The second cluster (C2) included three or more subclusters with the existence a relation among them. The first subcluster consisted of 3.0 accessions (AC09, AC16 and AC25), all of them were cultivars, and had mid-season ripening periods. As well as, AC16 and AC25 had yellow skin colors and maroon pulps. While, AC09 had a light red skin color, pink pulp and oblate fruit shape, with AC25, only AC16 had globose fruit shapes. The second cluster comprised of 4.0 accessions (AC13, AC14, AC19 and AC20), all of them are cultivars and mid-season ripening periods except AC13 with a late ripening period. The accessions had different skin colors and different pulp colors. While AC20 had a globose fruit shape, the other was oblate. The third subcluster consisted of 4.0 accessions (AC10, AC11, AC12 and AC15), AC12 and AC15 were wild types, the others were cultivars, oblate fruit shape, only AC15 was globose shaped fruit, with different skin and pulp colors. The third cluster (C3) contained 2.0 accessions (AC16 and AC17), both of them had yellow green skin colors, maroon pulp, oblate fruit shape as well as, mid-season fruit ripening periods and cultivar types. The fourth cluster (C4), comprised of the most of accessions, 41 accessions and about 7.0 subclusters, with different fruit skins and pulp colors as well as, different fruit shapes, all of them were cultivar types excluding AC37 and AC44, which are wild types. The fifth cluster (C5) included only one accession (AC49), it was brown skin color, amber pulp, and oblate fruit shape as well as had a mid-season ripening period and of cultivar type.

Table 7. Distance between clusters on centroids of fig accessions based on SCoT marker.

Clusters	C1	C2	C3	C4	C5
C1	0	3.65	5.46	3.44	7.08
C2		0	6.02	3.52	7.62
C3			0	5.85	8.14
C4				0	6.30
C5					0



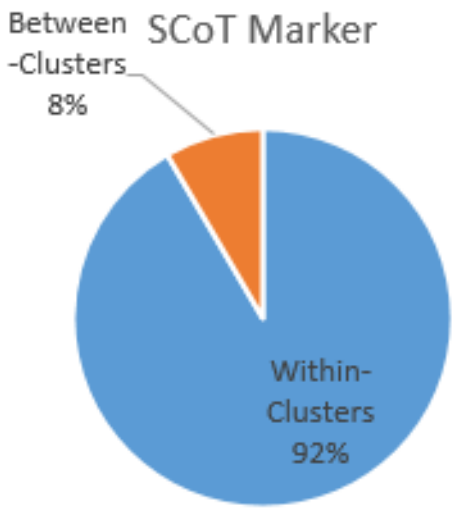


Figure 4. Determined variation within and among clusters of fig accessions

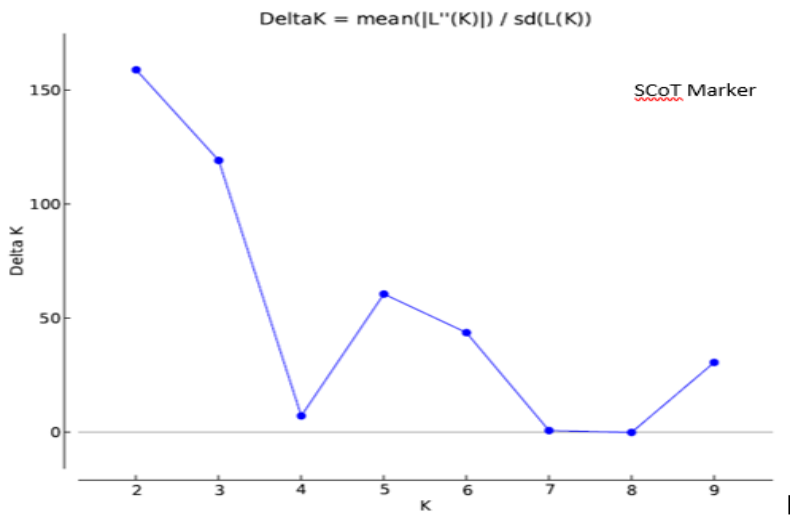
Figure 3. UPGMA dendrogram illustrated fig accessions clustering based on SCoT primers evaluation.

Assessment of population structure among fig accessions

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 (30). The results of assessing the 66 fig accessions by 15 SCoT primers were determined to admixture model-based reproductions with K ranging from 1.0 to 9.0. Depending on the maximum likelihood (77%) and K value, among the fig accessions, 2.0 major groups could be distinguished, when analyzed with 15 SCoT primers (Figure 5). The first group was red color consisted of 25 fig accessions within

different morphological and pomological traits. The second group comprised of 36 accessions, as well as, the accessions had various morphological and pomological characters, the remaining 5.0 accessions (AC15, AC20, AC32, AC41 and AC65), considered as the admixture between 2.0 populations, showed to have ancestry from more than one population, only AC44 was a wild type. Which indicates to the wild-type gene pool of the other fig cultivars. The results are confirmed by the results of (40) in that the structure software and delta K mean (K=2) separated 94 laurel genotypes into main populations, with10 SCoT primers. ΔK value accounted for all groups indicated a strong signal for K=2, depending on it and structure analysis, 278 individuals from 10 *Prunus sibirica* L., classified into 2.0 main populations with 23 SCoT primers (6).

The SCoT marker data between 2.0 populations (Table 8) inferred that the clusters value differed from 0.576 to 0.424, and predicted heterozygosity value ranged from 0.293 to 0.308, with an average of 0.3, as well as, fixation index value ranged from 0.174 to 0.142 with a mean of 0.158.



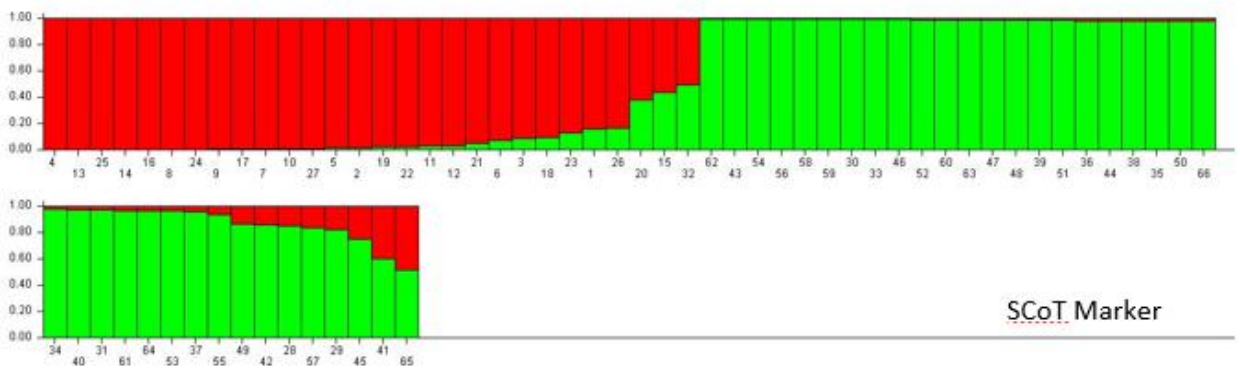


Figure 5. Sixty- six accessions of fig based on SCoT data clustered into 2.0 subpopulations. Population structure of fig accessions at K = 2.0. Each color represents a specific population subset. The horizontal axis numbers (1–66) correspond to the individual codes of fig accessions.

Table 8. A structure illustrating of 66 fig accessions, inferred clusters, expected heterozygosity and fixation index values.

Population	Inferred clusters	Expected heterozygosity	Fixation index
Population 1	0.576	0.293	0.174
Population 2	0.424	0.308	0.142
Mean	0.5	0.3	0.158

Conclusion

In essence, the present study showed that the fig accessions existing in mentioned province had high significant diversity when detected by phytochemical substance analysis and molecular marker (SCoT). The accessions revealed high ranges of SSC, TPC, TFC, AT and TSS compounds in whole fruits. The dark accessions had more antioxidant activity than the light one. Furthermore, accessions were classified into about 7.0 relative groups. The SCoT marke referred repeatable and reliable marker, as well as, more effectiveness to fig accessions discrimination. Generate high number of alleles, PIC, MI and polymorphic percent, divided the 66 fig accessions into 5.0 main clusters and numerous sub clusters based on similarity and dissimilarity among them.

However, the population structure classified accessions into 2.0 subpopulations based on K value and high range of probability. It was concluded that there is a richness source of fig germplasm, at the wild and cultivar shapes. It is very interested to find new varieties by breeding program.

Acknowledgement

This study was obtained from the doctoral dissertation, conducted in the University of Sulaimani, College of Agricultural Engineering Sciences / Horticulture Department. The authors would like to express their gratitude to the Department of Horticulture at the College of Agricultural Engineering.

Conflict of interest

The authors declare that they have no conflict of interest.

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