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Genetic diversity of Bottle gourd (*Lagenaria siceraria* (Molina) Standl.) landraces in Jordan assessed by Agro-morphological traits and Inter Simple Sequence Repeat markers

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Abstract

Bottle gourd (*Lagenaria siceraria* (Molina) Standl.) is an important crop in Jordan, but it remains under-researched. The primary aim of this study was to evaluate the genetic variability existing among different bottle gourd landraces in Jordan using Agro-morphological traits and molecular markers. Bottle gourd landraces were collected from various locations in Jordan; phenotypic differences and genetic variation using ISSR were studied between collected landraces. Results show that phenotypic coefficients of variance were larger than their genotypic coefficients of variance for all characters, demonstrating that the environment had an impact on these features. High genetic advance was found for plant length, number of leaves, leaf width and seed area; this could be explained by additive gene action. High heritability >60% estimates were observed. Principal component analysis identified two principal components responsible for 69.6% of total variation. Plant height showed positive association with number of tendrils, number of leaves and leaf length. 246 amplified markers were obtained using 24 ISSR primers, 135 of which were polymorphic. Genetic distance varied between 0.78 to 0.92 based on molecular analysis and ranged from 0.42 to 0.91 for agro-morphological data. Dendrograms constructed based on morphological and ISSR data clustered landraces to six and five main groups, respectively. The results of this work could be used in future bottle gourd breeding programs.

Keywords: Bottle gourd, genetic diversity, landrace, agro-morphological traits, ISSR.

1. Introduction

Bottle gourd (*Lagenaria siceraria* (Molina) Standl.), a member of the family Cucurbitaceae, having chromosome number 2n = 22, is a climbing, monoecious plant, with large white flowers, hairy stems, and long forked tendrils. Its fruits are fleshy and vary in shape and size. As archaeological evidence indicates that bottle gourd is one of the earliest plant species that have been cultivated for human uses (more than 10,000 years ago). Bottle gourd is grown worldwide for its fruit and for its medicinal value because it has essential constituents that are required for human health (Xu et al., 2011). Bottle gourd fruits were used traditionally as a cardio-protective, to promote diuresis, and to counteract poisoning (Prajapati et al., 2010). The ethanolic extract of this fruit was shown to be an effective antioxidant, hepatoprotective and cardiotonic agent (Deshpande et al., 2008). In addition to its nutritional and medicinal values, it has traditional and decorational uses (Mladenović et al., 2012). Bottle gourd seeds are also a valuable source of amino acids and essential oil (Ogunbusola et al., 2010). Another recent and useful agronomic application of bottle gourd is that it has been shown that bottle gourd rootstock is tolerant to low soil temperature stress and soil-borne diseases (Mashilo et al., 2016).

Landraces are defined as cultivar with unique characteristics and historical background, genetically diverse, having high adaptation potential, and with minimum genetic improvement (Villa et al., 2005). These landraces can adapt to shifting environmental conditions and modifying agricultural practices due to the genetic diversity inherent within their genome (Azeez et al., 2018).

Molecular markers are now being utilized in a variety of plant biodiversity studies. There are various molecular markers that can be used for genetic studies such as Simple Sequence Repeat (SSR), Random Amplified Polymorphic DNA (RAPD), Amplified Fragment Length Polymorphisms (AFLP) and Inter Simple Sequence

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Repeat (ISSR) (Idrees and Irshad, 2014). Molecular markers techniques are not influenced by environmental factors, stable across different developmental stages, and result in a powerful data regarding genetic distance and genetic similarity (Mashilo et al., 2016).

ISSR has the advantage of not requiring sequence knowledge neither for primer construction nor genome sequence information. It causes multi-loci and highly polymorphic patterns and is randomly distributed throughout the genome (Jabbarzadeh et al., 2010). ISSR markers have been demonstrated to have advantages over other genetic markers, including being ubiquitous, inexpensive, quick, simple to use, highly reproducible, and polymorphous (Mohammadabadi et al., 2017). ISSR method has been used in genetic diversity studies in various crops, including strawberry, potato, rice and others (Tahir and Karim, 2011; Brake et al., 2021).

The objective of this study was to assess the biodiversity status of bottle gourd landraces collected from different regions of Jordan using molecular and agro-morphological markers.

2. Materials and Methods

2.1. Plant materials

Nineteen *Lagenaria siceraria* landraces were used in this study. Eleven landraces were collected from different regions in Jordan, and eight samples were provided by the National Agriculture Research Centre (NARC) (Table 1). Landraces seeds were grown in the field at Yarmouk University/ Irbid during the period of February – August, 2020 under rainfed conditions. The study site has silty clay soil texture. Three replications were used with 15 seeds in each replicate. Field was kept free of weeds and diseases during the experiment.

Table 1: Bottle gourd landraces and description of collection sites

Number #	Sample code	Location	Altitude	Longitude	Latitude
1	4414	Irbid	620	N 35.85	E 32.55
2	4451	Irbid	620	N 35.85	E 32.55
3	4514	Irbid	620	N 35.85	E 32.55
4	4515	Irbid	620	N 35.85	E 32.55
5	4517	Irbid	620	N 35.85	E 32.55
6	4597	Irbid	620	N 35.85	E 32.55
7	-	Ibbin	1141	N 32.37	E 35.82
8	4460	Balqa'	546	N 35.75	E 23.12
9	851	Balqa'	546	N 35.75	E 23.12
10	-	Madaba	709	N 31.42	E 35.47
11	-	Al-Karak	930	N 31.10	E 35.42
12	-	Aqaba	6	N29.30	E 35.00
13	-	Sal	574	N 32.34	E 35.54
14	-	Al-mazar	1264	N 35.65	E 31.82
15	-	Samaalrousan	350	N 31.55	E 35.55
16	-	Kufrjayez	508	N 23.37	E 35.49
17	-	Baytyafa	632	N 32.52	E 35.78
18	-	Deirabe said	508	N 23.30	E 35.41
19	-	Enbeh	541	N 31.96	E 35.93

2.2. Agro-morphological traits analysis

Seeds of each landrace were planted at a depth of 25 mm, spaced 60-70 cm between plants. A completely randomized design was laid out with three replicates. For agro-morphological traits assessment, several traits were measured including; seed length, seed width, seed area, plant length, leaf length and width, number of leaves, number of tendrils, length of tendrils.

2.3. ISSR analysis

Young bottle gourd leaves were collected from all landraces and DNA was extracted using CTAB method (Doyle, 1991). A total of 24 ISSR primers (University of British Columbia, Canada) were used (Table 6). DNA amplification was performed in Genepro (model- TC-E-96G) thermal cycler. PCR reactions were carried out in a final volume of 25 µl containing30 ng of genomic DNA, 12.5 µl of 1X master mix,1.5µLof each primer and 9 µl free nuclease water. The PCR program consists of an initial denaturation step of 94ºC for 5 min, followed by 40 cycles of 240 s denaturation at 94ºC, 300 s annealing at 50ºC, and 60 s extension at 72ºCwith final extension step at 72ºC for 5 min. Amplified products of ISSR were separated by electrophoresis using 1.25% agarose gel stained with ethedium bromide and visualized using gel documentation system (Alpha DigiDoc System, USA).

2.4. Statistical analysis

The whole experiment was repeated two times. The collected data were processed using one-way analysis of variance (ANOVA). Investigation of multi-character variation was conducted by Cluster Analysis and Principal Component Analysis (PCA). Dendrogram for molecular and agro-morphological

data was drown using NTSYS pc (2.20) Software. ImageJ software was used to measure seeds length, width and area for each landrace. The DNA profile was scored from the resulting gels. Clear bands were considered as a separate marker and scored as either present (1) or absent (0) across all landraces. Primer efficiency and primer discrimination were calculated by dividing the number of markers for each primer by the total number of markers and by dividing the number of polymorphic markers by the total number of polymorphic markers, respectively (Khierallah et al., 2011). DNA fragment size was estimated using the relative migration distance of DNA ladder.

3. Results

3.1. Agro-morphological characterization

Table 2 displays the mean and standard error of studied landraces morphological characteristics. The quantitative features examined showed significant differences (P< 0.05) among landraces, demonstrating the presence of morphological variations. For example, sample number 8 collected from Balqa', showed the longest plant length (2.05 m), highest number of tendrils (11) and highest number of leaves (14). On the other hand, sample number 15 collected from Samaalrousan, showed the shortest plant length (0.35 m),samples 14 and 19 showed the lowest number of tendrils, and samples 1 and 19 showed the lowest number of leaves.

Table 2: Mean and standard error for quantitative traits of 19 bottle gourd landraces.

Sample number	Plant length (m)	Number of tendrils	Tendril length (cm)	Number of leaves	Leaf width (cm)	Leaf length (cm)	Distance between tips of cotyledons (cm)	Petiole length (cm)	Seed length (cm)	Seed width (cm)	Seed area (cm^2)
					Mean ± SE						
1	1.08±0.04	4±0.9	21±1.1	6±1.0	9.75±1.2	8.5±1.5	12.5±1.1	5.5±0.2	1.6±0.0473	0.75±0.01	1.11±0.03
2	1.3±0.02	8±0.7	15±0.9	11±0.8	13±1.1	10.5±0.8	15±0.9	5±0.4	1.8±0.11	0.7±0.01	1.21±0.02
3	2.2±0.08	9±0.5	22±1.2	13±0.9	14.5±1.2	14.5±1.1	14±1.2	6±0.4	1.8±0.05	0.78±0.01	1.23±0.01
4	1.65±0.05	6.5±0.5	36±1.0	11±1.1	15±1.5	11±0.4	15±1.1	7.5±0.5	1.9±0.08	0.7±0.02	1.30±0.05
5	1.07±0.08	4.33±0.5	25.3±1.3	7±0.5	9.75±0.6	7.9±0.3	12.8±1.4	5.6±0.4	1.6±0.03	0.8±0.02	1.26±0.05
6	1.25±0.29	8±0.6	25.5±0.5	10.5±1.1	11.5±0.5	11.5±0.5	14.5±1.5	7±0.3	1.6±0.05	0.57±o.o3	0.91±0.02
7	0.97±0.01	4.66±0.6	28.3±1.3	8±1.1	8.6±0.8	7.6±0.8	12.3±1.7	5±0.2	1.7±0.02	0.65±0.02	1±0.01
8	2.05±0.04	11±0.9	29±1.2	14±0.8	12.5±0.9	11±0.9	14±0.9	5±0.3	1.8±0.05	0.69±0.01	1±0.09
9	1.11±0.09	6.66±0.8	12±1.4	9±0.8	9.33±0.6	8.3±0.7	15.6±1.8	4.75±0.2	1.7±0.02	0.76±0.02	1.1±0.03
10	0.82±0.1	4.5±0.6	23.3±1.0	8±0.7	8.33±0.6	7.4±0.6	16.25±0.9	4.8±0.4	1.6±0.04	0.65±0.01	0.94±0.05
11	0.79±0.02	5±0.5	22±0.5	8±0.5	9.25±0.5	8.5±0.5	14±1.1	6.3±0.4	1.5±0.08	0.86±0.01	1.1±0.06
12	1.97	9±0.8	19±0.8	13±0.8	12.5±0.7	10.5±0.8	15±1.3	7±0.5	1.6±0.08	0.74±0.02	1.2±0.05
13	0.51±0.09	4.75±0.5	28.3±1.6	7.75±1.3	10±0.3	8.5±0.4	11±0.8	6±0.4	1.6±0.06	0.63±0.02	0.95±0.02
14	0.91±0.06	3±0.4	22±0.5	6.3±0.6	10.1±0.6	9.2±0.4	14.3±0.8	6.8±0.4	1.6±0.02	0.79±0.03	1.0±0.04
15	0.35±0.06	5±0.3	23.5±0.5	7.5±0.5	9.25±0.2	7.75±0.3	14±1.0	3.75±0.25	1.6±0.04	0.67±0.01	0.94±0.01
16	0.91±0.06	3.66±0.3	27.2±1.8	6.4±0.8	8.8±1.0	7.7±0.5	12.4±0.8	5±0.3	1.5±0.06	0.65±0.03	0.88±0.04
17	0.64±0.03	8±0.4	21±1.1	11±0.7	9±0.8	9.5±0.4	14±1.1	5±0.5	1.9±0.05	0.79±0.02	1.36±0.06
18	1.2± 0.07	8.5±0.5	19.5±1.5	19±0.5	16.5±0.5	12.5±0.5	13.3±1.2	5.5±0.7	1.5±0.03	0.67±0.03	0.87±0.03
19	1.65±	3±0.5	23.5±1.4	6±0.5	9.16±0.7	8.8±0.3	13.5±0.6	4.8±0.2	1.7±0.06	0.70±0.01	1.0±0.03

3.2. Genetic parameters of agro-morphological traits

Estimates of genetic advance (GA) and genetic advance as a percentage of the mean (GAM), genotypic variance (Vg) and phenotypic variance (Vp), genotypic coefficient of variation (GCV), phenotypic coefficient of variation (PCV), broad sense heritability (H2), and genetic advance (GA) are presented in Table3. High phenotypic and genotypic variances were observed for tendril length (33.3 and 15.2, respectively). On the other hand, small genotypic and phenotypic variances were observed for seed width (0.006 and 0.007) and seed length (0.01 and 0.02).

In general, the relative coefficients values indicate the level of variability existing in a population. Genotypic and phenotypic coefficients of variation are straightforward measures of variability. Low magnitude of genotypic as well as phenotypic coefficient of variation were observed for number of tendrils (1.48 and 2.02, respectively). Moderate GCV and PCV were observed for leaf width (16.4 and 21.0), leaf length (14.1 and 18.6), petiole length (12.7 and 18.9), seed width (10.6 and 11.4) and seed area (13.2 and 14.9).

Broad sense heritability (H^2) ranged from 41.98% to 85.7%, for distance between cotyledons and seed width, respectively. Genetic advance is an increase in a plant's mean genotypic value relative to its parent population, the highest value of GA was observed for tendril length (5.42) and the lowest (0.05) for distance between cotyledons. The highest genetic advance as per cent of the mean (GAM) (47%) was recorded for plant length, and the lowest percent was observed (2%) for tendrils number (Table 3).

Table 3: Estimates of genetic parameters for 11 quantitative traits in different bottle gourd landraces.

	Plant length	Number of tendrils	Tendril length	Number of leaves	Leaf width	Leaf length	Distance between cotyledons	Petiole length	Seed length	Seed width	Seed area
Between groups	0.38	11.0	63.7	23.9	11.0	6.76	5.77	2.15	0.05	0.01	0.07
Within group	0.05	2.07	18.2	3.26	2.08	1.30	5.60	0.62	0.009	0.001	0.006
Mean	1.19	116.6	23.3	9.60	10.9	9.53	13.9	5.6	1.70	0.73	1.089
Vg	0.11	2.99	15.2	6.89	3.17	1.81	0.06	0.51	0.01	0.006	0.02
Vp	0.16	5.05	33.3	10.1	5.25	3.13	5.67	1.13	0.02	0.007	0.03
GCV	27.6	1.48	16.7	27.3	16.4	14.1	1.70	12.7	6.69	10.6	13.2
PCV	33.4	2.02	24.7	33.2	21.0	18.6	17.2	18.9	8.70	11.4	14.9
H^2	67.9	59.1	45.5	67.9	60.4	58.1	41.98	45.02	59.0	85.7	77.5
GA	0.56	2.87	5.42	4.46	2.85	2.12	0.05	0.99	0.1	0.15	0.26
GAM (GA %)	47	2	23	46	26	22	36	17	5	20	24

3.3. Principle component analysis

Principal component analysis was used to detect the most significant variables in the data set. It measures the importance and contribution of particular trait relative to the total variability. Table 4 displays the eigen values and principal components (PCs) for quantitative qualities. For 19 landraces, the first two principal components (PCs) with eigenvalues greater than one explained 69.6% of the variation in various agro-morphological parameters. Other PCs had eigenvalues less than 1. The first principal component (PC1) accounted for 50.8% of total variation. Quantitative traits that contributed more positively toPC1 included plant height, number of tendrils, number of leaves, leaf width, leaf length, distance between tips of cotyledons, petiole length, seed length, seed width, seed area, whereas tendril length was negatively associated with PC1. Principal component 2 (PC2) showed 18.8% of the total agromorphological variability. Plant height, number of tendrils, number of leaves, leaf width, leaf length, distance between tips of cotyledons, petiole length, seed length, seed width, seed area contributed positively to PC2, whereas tendril length was negatively associated with PC2 (Figure 1). Plotting the link between 19 bottle gourd landraces using the first two PCs (Figure 2). This distinction between the landraces was made on the basis of significant morphological variations.

Table 4: Eigen value and percent of total variation for the principal component axes.

	PC1	PC2
% of variance	50.753	18.822
Eigenvalue	5.583	2.070
Cumulative %	50.753	69.575

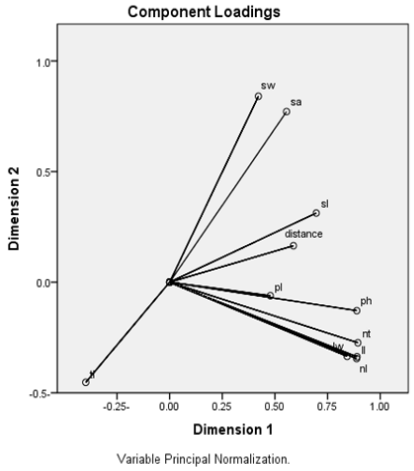

Figure 1: Scattered diagrams of first two principal components showing the contribution of various traits in the separation of various *Lagenaria siceraria* landraces from Jordan. (sw:seed width; sa: seed area; sl:seed length; distance:distance between cotyledons; pl:petiole length; ph:plant length; nt:number of tendrils; ll:leaf length; lw:leaf width; nl:number of leaves; tl:tendril length).

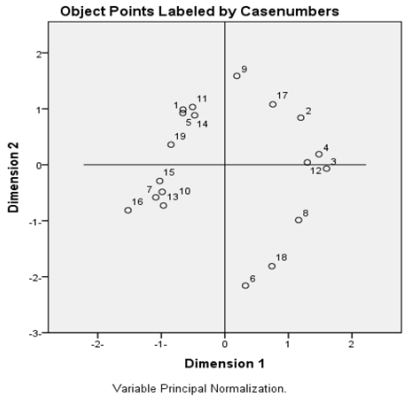

Figure 2: Scattered diagram of first two principal components based on mean values of 19 quantitative traits in *Lagenaria siceraria* of bottle gourd from Jordan.

3.4. Correlation analysis of agro-morphological traits

Correlation assesses the degree of association between variables. The results of correlation analysis (Table 5) in the present study showed a positive strong correlation between plant length and number of tendrils (r=0.86), number of leaves (r=0.67) and leaf length (r=0.74). On the contrary, tendril length had negative correlation with distance between tips of cotyledons (r=-0.36) and number of leaves (-0.12). Furthermore, a positive correlation was observed between number of leaves with leaf length and width(r=0.79) (r=0.83) respectively. In addition, a positive correlation between plant length with seed length (r=0.56), seed width (r=0.10) and seed area (r=0.48) was observed.

Table 5: Correlation coefficients among morphological traits in bottle gourd landraces.

	Number of tendrils	Tendril length	Number of leaves	Leaf width	Leaf length	Distance between the tips of cotyledons	Petiole length	Seed length	Seed width	Seed area
Plant length	0.86	0.07	0.68	0.65	0.74	0.20	0.28	0.56	0.10	0.48
Number of tendrils		-0.14	0.86	0.66	0.75	0.33	0.13	0.45	-0.07	0.28
Tendril length			-0.12	0.05	0.07	-0.36	0.29	0.02	-0.19	0.09
Number of leaves				0.83	0.79	0.23	0.17	0.21	-0.11	0.08
Leaf width					0.88	0.17	0.43	0.25	0.01	0.16
Leaf length						0.19	0.42	0.34	0.08	0.21
Distance between the tips of cotyledons							0.09	0.27	0.06	0.28
Petiole length								0.04	0.22	0.25
Seed length									0.15	0.71
Seed width										0.78

3.5. Dendrogram for agro-morphological traits

The collected landraces were clustered into six main groups as shown in agro-morphological traits Dendrogram(Figure 3). The first group comprises 4414-Irbid, Ibbin, 4517-Irbid, Al-karak, Samaalrousan, Bayt yafa, 4597-Irbid, 4515-Irbid, Madaba, 851-Balqaa'. The second group includes 4451-Irbid only. Two landraces (Aqaba and 4460-Balqaa') were grouped in group 3. Group4 includes Al-mazar, Enbeh and Kufrjayez, while group 5 includes 4514-Irbid only, and finally group six contains landrace collected from Deirabe said region.

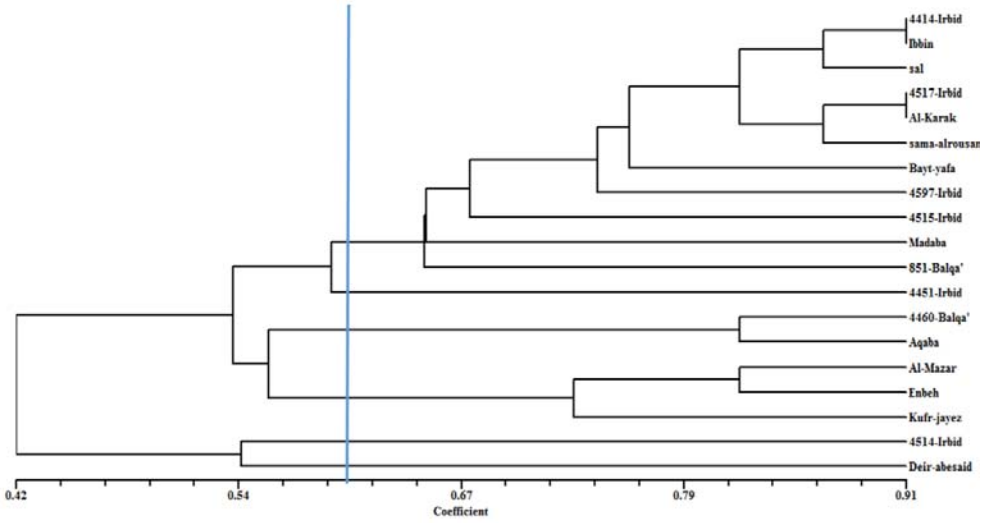

Figure 3: Dendrogram constructed from Agro-morphological data.

3.6. ISSR analysis

The selected primers and the maximum number of reproducible polymorphic bands produced by each primer, % Primer efficiency, number of Polymorphic bands, number of unique bands, % of polymorphism, %Discrimination power and PIC are listed in Table 6. A total of 246 markers were produced by 24 primers, of which 135 were polymorphic. The efficiency of ISSR primers has been tested in producing polymorphic DNA bands in the bottle gourd genome, the highest primer efficiency was 8.53 for UBC-857 and the lowest was 0.81 for UBC-845, and the average primer efficiency for all primers was 4.43%. A 100% polymorphism was found for UBC-825, and 0%was observed for UBC-812, UBC-864 and UBC-844. The average percentage of polymorphic markers for all primers in the examined 19 landraces was 54%.

The discrimination power ranged from 0% (for UBC-864, UBC-812 and UBC-841) to 12.5% (for UBC-856 and UBC-857). Primers UBC-823, UBC-843, UBC-856, UBC-860, UBC-828 and UBC-815 were able to generate 2, 1, 1,1,1,1 unique bands, respectively.

For cluster analysis, Jaccard similarity coefficients were used. Dendrogram was formed using Unweighted Pair-Group method (UPGMA) by SAHN clustering function of NTSYS software. The dendrogram's similarity coefficient ranged from 0.74 to 0.92. (Figure 4). Five main groups of the investigated landraces were identified: Group 1 comprises 4414-Irbid, 4451-Irbid, 4514-Irbid, 4517-Irbid, 4597-Irbid, Ibbin, 4460-Balqaa' and 851-Balqaa'. Eight landraces were found in group 2; Al-Karak, Aqaba, Samaalrousan, Kufrjayez, Baytyafa, Enbeh, Sal, Al-Mazar. Group 3 comprises 4515-Irbid only, group 4 contains only Madaba, and finally group 5 contains deirabesaid only.

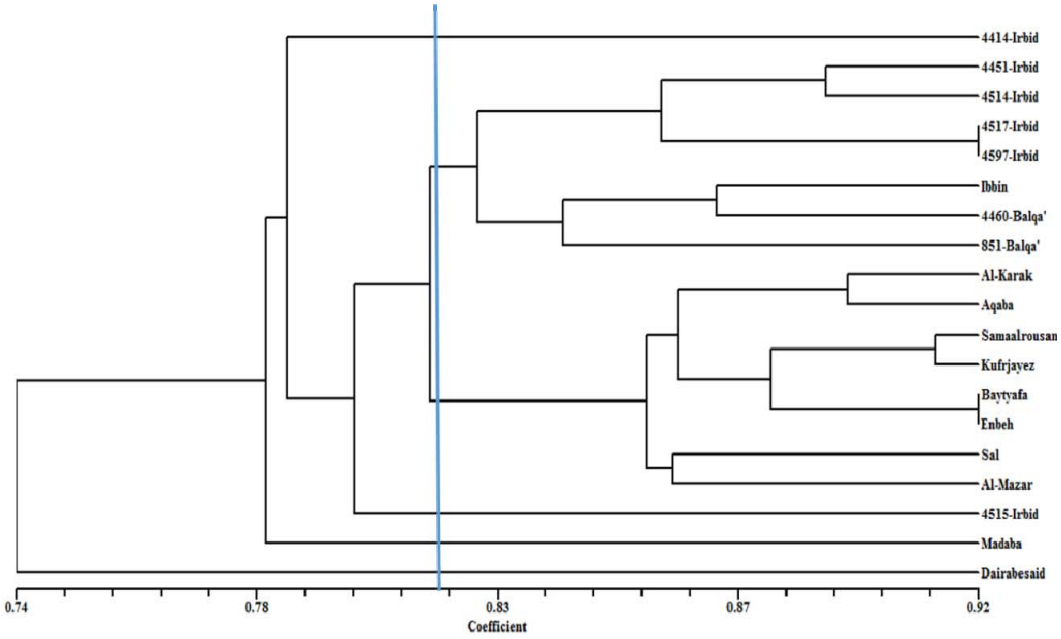

Figure 4: Dendrogram estimating the genetic distance among 19 bottle gourd landraces based on ISSR data.

Table 6: Primer name, total number of markers generated, % Primer efficiency, number of Polymorphic bands, number of unique bands, % of polymorphism, %Discrimination power and PIC.

Primer name	Total number of bands	%Primer efficiency	Polymorphic band	Unique band	% Polymorphism	% Discrimination power	Polymorphic Information Content (PIC)
UBC-807	14	5.69	8		57.14	5.92	0.26
UBC-823	11	4.47	8	2	72.72	5.92	0.61
UBC-826	11	4.47	6		54.54	4.44	0.34
UBC-843	6	2.43	4	1	66.66	2.96	0.68
UBC-848	11	4.47	7		63.63	5.18	0.35
UBC-856	19	7.72	17	1	89.47	12.5	0.77
UBC-880	13	5.28	2		15.38	1.48	0.15
UBC-900	7	2.84	5	1	71.42	3.70	0.44
UBC-812	13	5.28	0		0	0	0.00
UBC-860	6	2.43	2	1	33.33	1.48	0.31
UBC-864	8	3.25	0		0	0	0.00
UBC-810	11	4.47	5		45.54	3.70	0.21
UBC-868	16	6.50	5		31.25	3.07	0.11
UBC-834	15	6.09	4		26.66	2.96	0.16
UBC-855	14	5.69	11		78.57	8.14	0.45
UBC-825	8	3.25	8		100	5.92	0.75
UBC-828	9	3.65	5	1	55.55	3.70	0.52
UBC-815	11	4.47	9	1	81.81	6.66	0.81
UBC-835	5	2.03	4		80	2.96	0.26
UBC-841	6	2.43	0		0	0	0.00
UBC-845	2	0.81	1		50	0.74	0.30
UBC-844	9	3.65	7		87.5	5.18	0.39
UBC-857	21	8.53	17		80.95	12.5	0.40
Total	246		135				
Avg.		4.43			54.00		

4. Discussion

Statistical analysis results (ANOVA) revealed significant differences between the tested landraces for all morphological characteristics, demonstrating the considerable variability among landraces. Similarly, variations in morphological characters have been reported in Turkish bottle gourd landraces. *Lagenaria siceraria* was found in 162 accessions, all of which were gathered from Turkey's Mediterranean coast. These accessions varied greatly in several morphological characteristics, particularly in fruit size and form (Yetişir et al., 2008). One of the major factors that influence the phenotypic characteristics in organisms is the environmental influence. According to Parsaeian et al.(2011), there is a high degree of variability in the phenotypic traits of 27 accession of sesame (*Sesamum indicum* L.) that were collected from different regions of Iran along with six exotic genotypes from Asian countries. They explained that this high degree of variability is caused by the effect of different environmental aspects on phenotypic features and the complex genetic structure of many morpho-physiological traits.

According to the current study, for each of the investigated features, phenotypic variance (Vp) and phenotypic coefficient of variance (PCV) were greater than their associated genotypic variance (Vg) and genotypic coefficient of variance (GCV).This suggests that there was significant interaction between the environment and these characters' expressions, which was greatly influenced by the surrounding conditions. Similarly, Konate et al., (2016) reported that rice (*Oryza sativa* L.) phenotypic coefficients of variance were higher than genotypic coefficients of variance in all the studied characters, which indicates environmental influence on traits expression. This, however, contrasts Malek et al(2014) study, they studied

genetic diversity among 27 soybean genotypes and found small differences between phenotypic and genotypic coefficients of variation (PCV and GCV) for most characters, which shows less environmental effect on these features' manifestation. In genetic studies of quantitative features, one of the most crucial roles of heritability estimation is its ability to forecast the accuracy of phenotypic value as a breeder's guide (Singh et al., 2011). In this study, high heritability values (>60%) for seed width, seed area, plant length, number of leaves and leaf width were observed. High heritability in morphological features suggests that these characters could be easily improved through selection (Rashid et al., 2017). Low heritability values, however, were found for the length of the tendrils, the number of tendrils, the length of the tendrils, the length of the leaves, the length of the petioles, and the length of the seeds. These characters with low value of heritability are of little importance in selection strategies because their most variations are non-transmissible (Singh et al., 2011).

According to Ajayi et al., (2014), genetic advance as a percentage of mean is divided into three categories: low(10%), moderate(10%–20%), and high (>20%). High genetic advance as percentage of mean was observed for plant height, tendril length, number of leaves, leaf width, leaf length and distance between tips of cotyledons, whereas number of tendrils, and seed length showed low genetic advance as percentage of mean and moderate genetic advance for petiole length and seed width.

Characters with high heritability coupled with high genetic advance as a percent of mean (GA %) resulted from additive gene action could be used in further improvement programs due to their performance and simple phenotypic selection (Reddy et al., 2013). In the current study, high heritability values coupled with high genetic advance were recorded for plant height, number of leaves, leaf width and seed area. Similar findings were reported by Kumar et al. (2016) where high heritability coupled with high genetic advance as percent of mean recorded for almost all of the characters they studied.

In this study, no strong association was observed between geographical origin of bottle gourd landraces and phenotypic traits, except for one group of landraces;Kufrjayez, Al-mazar and Enbeh were in one cluster, and they belong to one geographical area (Irbid governorate). All the other landraces were clustered according to morphological traits rather than to geographical distribution. This is in agreement with the results of Pandey et al. (2015) who found that clustering of sesame genotypes based on their morphological traits did not reflect their geographical origin. In plant breeding, correlation assists in the choice of characters whose selection would result in the improving of any character such as yield (Joshi, 2005). In this study, a positive strong correlation between plant length and number of tendrils (r=0.86), number of leaves (r=0.67) and leaf length (r=0.74) was found. This indicates that plant length is a helpful characteristic to bottle gourd breeders because it is associated with a high number of tendrils and high number of leaves. Similarly, research has indicated that one of the most crucial qualities taken into account in the development of high yield variants in plant breeding projects is plant length (Ahmad et al., 2015). On the contrary, tendril length had negative correlation with distance between tips of cotyledons (r=-0.36) and number of leaves (-0.12). Moreover, there was a positive correlation between number of leaves with leaf length and width. Leaf area is considered as an important parameter in determining plant growth and it has an important role in yield and productivity (Hashimoto et al., 2023). Tendrils are considered as specialized stem, leaf or petiole with a threadlike shape that plants used for climbing, support and attachment; high number of tendrils led to an improvement in standing ability of crops (Liou and Ruan, 2011).

ISSR and other molecular markers are powerful tools for genetic characterization of bottle gourd (Decker-Walter et al., 2001; Abdinet al., 2014). In this study, the average percentage of polymorphic markers for all primers in the examined 19 landraces is 54%, which indicated a high level of genetic variation among landraces. Similar findings were reported by Yuan et al.(2015) who examined the genetic variety of 48 Okra (*Abelmoschus escullentus* L.) genotypes using morphological features and ISSR markers. Their study found that the average polymorphism was 54.55%. The greatest similarity coefficient between the landraces Enbeh and Baytyafa was 0.92; this shows a significant degree of resemblance between them and suggests that they may have common ancestor. Landraces 4515-Irbid and Dairabe said showed the lowest similarity coefficient values (0.68), indicating that they are distinct from one another due to the diverse morphological characteristics and different geographic origins or ancestors. Based on ISSR marker analysis, the studied landraces cluster into various groups, indicating that they are genetically distinct. Landraces placed in the same groups represent a close genetic similarity. There was no clear association between genetic divergence and geographical origins in the same cluster. One explanation for this could be the limited number of ISSR markers used. Izadpanah et al. (2015) reported similar findings, no correlation between Saffron's(*Crocus sativus L.*) diversity pattern and its geographic origins. In the present study, no full agreement was observed between molecular and agro-morphological analysis except for 8 landraces which grouped similarly in both dendograms; namely, Al-mazar, Enbeh and Kufrjayez were found in one group in both dendograms, and 851-Balqaa', Ibbin and 4414-Irbid in another group in both dendograms also. Finally, deirabesaid was found in a distinct group in both dendograms, which is in agreement with studies of Greene et al. (2004), Yuan et al., (2015) and Guliyev et al., (2018) who used morphological and molecular markers in red clover (*Trifolium pratense* L.), okra (*Abelmoschus escullentus* L.) and melon (*Cucumismelo* L.); they

found that there is no association between molecular and morphological analysis in terms of clustering. Garciaet al. (2008) found that there is no correlation between morphological and RAPD methods in many strawberry varieties cultivated in Argentina. Moreover, Ristiet al. (2013) investigated the genetic diversity of maize landraces using 15 morphological features, 7 RAPD primers, and 10 SSR primer pairs, and they found that no significant associations between any two types of markers were detected. Syed (2016) proposed two explanations for the weak correlation between DNA fingerprinting markers and morphological characters. The first one is the possibility that a few alleles may be responsible for morphological characteristics and that their genotypes may not be correlated with the overall marker scores for these lines. Also, molecular markers study relatively most of the genome, which is wider than the morphological markers only.

In conclusion, ISSR markers were significantly valuable for genetic diversity analysis at the molecular level in bottle gourd landraces. They showed high discrimination power, and were able to differentiate between bottle gourd landraces with high efficacy and accuracy**.** The findings imply that there is significant genetic diversity at both the agromorphological and molecular levels. This shows the possibility of such landraces in bottle gourd breeding initiatives. Despite this, ISSR markers did not completely match agro-morphological data. Only 8 landraces were grouped similarly in both dendograms. Neither molecular nor agro-morphological diversity was associated with geographical distribution of landraces in Jordan.

5. Conflict of Interest

The authors declare that there are no conflicts of interest.

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