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Full Length Article

Assessment of Cowpea (*Vigna unguiculata*) Genetic Diversity using ISSR Markers, Biochemical Content and Agro-Morphological Traits

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Abstract

Cowpea is an essential crop for millions of people worldwide. This study was designed to assess cowpea landraces collected from various parts of Jordan using inter-simple sequence repeat markers, biochemical content and agro-morphological traits. Molecular analysis results show that twenty-three ISSR primers produced 259 markers, with an average number of markers of 11.26 for each primer. UBC835 primer produced the highest number of markers (19 markers) and the highest primer efficiency (8.48). Primers UBC815, UBC899, UBC864 and UBC810 generated two, one, one and one unique bands, respectively. Genetic similarity values ranged from 0.46 to 0.86, with an average of 0.67. The dendrogram illustrates that samples are clustered in two main groups. Biochemical content analysis showed significant variation between collected landraces, moisture content ranged from 3.2 to 5.1%, total phenolics content ranged from 10.2 to 15.2 mg/100 g and protein content ranged from 19 to 26%. Agro-morphological trait analysis shows significant variation between collected landraces, especially in plant height (ranging from 9.33 cm to 36.66 cm). The highest phenotypic coefficient of variance was recorded for seed width (50.27), while the highest genotypic coefficient of variation was recorded for seed width (49.16). The highest heritability value was recorded for seed width (98.06). Landraces were separated into three main groups based on agro-morphological traits. Overall, neither genetic nor agro-morphological dendrograms were related to geographical distribution.

Keywords: Landraces; Inter-simple sequence repeat markers; Genotypic variation; Phenotypic variation; Heritability

Introduction

Cowpea [*Vigna unguiculata* (L.) Walp.] is herbaceous climbing annual plant with an external shape similar to the common bean. Cowpea leaves are dark green and shiny, and the root system is branched (Timko *et al.* 2007). Cowpea is a diploid plant (2n = 2X = 22 chromosome) that belongs to Fabaceae family (Iwata-Otsubo *et al.* 2016) and is a stable pulse crop. Cowpea is a highly adapted crop cultivated in the same area to harsh environmental conditions, such as high temperatures, drought, and alkaline and acidic soil conditions (Ehlers and Hall 1997; Hall *et al.* 2002).

Cowpea is an essential crop for millions of people worldwide. The tremendous nutritional value and the ability to adapt to harsh environmental conditions make cowpeas a familiar crop for millions worldwide (Osipitan *et al.* 2021; Mekonnen *et al.* 2022). According to the FOW organization, the world production of cowpeas in 2021 was 8986191 tons, growing on 14911307 hectares and yielding 6026 100 g/hectares (FAOSTAT 2023). Numerous studies have shown that cowpea has a high nutritional value, with two to four times more protein than cereal and tuber crops. Additionally, it has relatively low-fat content (Devi *et al.* 2015; Jayathilake *et al.* 2018; Abebe and Alemayehu 2022). Due to the cost of meat and fish, people in developing countries focus on grain legumes as a source of protein (Rebello *et al.* 2014).

Landrace is mainly used to describe a cultivated plant with a historical origin and definite identity. It is locally adapted and linked with traditional farming systems (Villa *et al.* 2005; Sababheh 2018). Landraces are essential in improving crop production (Marone *et al.* 2021). The selection and improvement of landraces occur by selecting plants with favorable traits and growing those plants in the following year; repetition of this process for hundreds of years helps to enrich the genetic pool of crops (Glaszmann *et al.* 2010). Farmers, scientists, and researchers highly trust landraces for many reasons. For example, most landraces

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are disease-resistant, tolerant to biotic and abiotic environmental factors, and have relatively high production yields. So, it plays an essential commercial role in food production worldwide (Berg 2009).

The Inter-simple sequence repeat (ISSR) is used to study the genetic variation between landraces because it is cheap, quick, simple, and has high reproducibility (Ramesh *et al.* 2020). Also, the primer used in ISSR is easy to design. The primer of ISSR should be one of the three following forms: (a) unanchored primer, which consists of the repetitive motif (e.g., 5'–(AC)8–3')), (b) 5'-anchored primer; this form consists of the repeated motif in 3' side (*e.g*., 5'–GA(AC)8–3'), (c) 3'-anchored primer; this form consists of the repeated motif with one or several non-motif nucleotides at the 3'-end, e.g., 5'–(AGC)8 TY–3 (Reddy *et al.* 2002). ISSR could reveal the intra and inter-genomic diversity within unique genome regions and is considered to have more potential than other genetic markers (Zietkiewicz *et al.* 1994).

In Jordan, about nineteen types of cowpea landraces are adapted to biotic and abiotic stresses in the Jordanian environment, such as drought, high salinity in some areas, alkalinity in others, and low soil fertility. To use these landraces in breeding programs as a source for new genes, the genetic diversity among these landraces must be investigated. Until now, there has been no characterization study on Jordanian cowpea landraces at the molecular or morphological levels; therefore, this study aimed to assess the degree of similarity and differentiation among cowpea landraces in Jordan through molecular and agro-morphological characterizations.

Materials and Methods

Plant materials and cultivation

Nineteen cowpea landraces were studied in this research. Fifteen landraces were provided as seeds by the gene bank in the National Agricultural Research Center (NARC, Jordan), and four landraces were collected from different parts of Jordan. All landraces' seeds were grown in loam soil at 24 C, and irrigated 3 times a week in the greenhouse at Yarmouk University for molecular, biochemical, and agro-morphological analysis. Fifteen replicates were used for each landrace. Table 1 shows the locality of the collected landraces. Six replications were used for each landrace with a completely randomized design (CRD).

DNA extraction

Plant genomic DNA was extracted from cowpea landraces manually using extraction buffer (20 mL 1 *M* tris-base (PH = 7.5), 5 mL 5 *M* NaCl, 5 mL 0.5 *M* EDTA and 5 mL 10% SDS with a final volume of 100 mL using distilled water). Firstly, 0.8 grams of premature leaves were ground with 500 μL of extraction buffer, vortexed for 10 s and centrifuged for 10 min at 10,000 rpm at room temperature. After that, 400 μL of the centrifuged product was mixed with 600 μL isopropanol. The mixture was then incubated at room temperature for 30 min. After incubation, samples were centrifuged at 10,000 rpm for 10 min and then the supernatant was removed. After that, 500 μL 70% ethanol was added, and the mixtures were centrifuged for 3 min at 10,000 rpm at room temperature. The centrifugation step was repeated three times. Finally, the supernatant was removed, and the sample dried for 10 min. 30 μL of autoclaved distilled water was added to the sample, incubated at room temperature for 30 min, and then stored at -20°C for further analysis.

ISSR-PCR analysis

Extracted DNA was amplified using the Genepro model-TC-E-96G thermal cycler. The total volume was 20 μL for each reaction. Each PCR reaction contains a 12.5 μL master mix, 4 μL nuclease-free water, and 1.5 μL primer. Twenty-four ISSR primers designed by Reddy *et al.* (2002) were used to study the genetic variation.

Agarose gel-electrophoresis

PCR products were separated by agarose gel electrophoresis. The PCR products were loaded on 1.5% agarose gel submerged in 1% TBE buffer. After that, run at 90 volts for 90 min. The gel was scored under ultraviolet light. Molecular amplification product sizes were estimated using a 150-bp DNA ladder.

Data scoring

The gel was analyzed distinctly for each primer by recording the absence or presence of all PCR fragments in individual lanes, where (1) referred to the presence of an amplified fragment and (0) to its absence. Moreover, (.) referred to a fragment that cannot be determined.

Biochemical analysis

Three biochemical characteristics were used in this research: moisture content, protein content, and total phenolic contents. The moisture content of cowpea landraces was determined as described by Kauth and Biber (2015). Protein and total phenolic contents were measured according to Alu'datt *et al.* (2020); briefly, 0.3 mL methanolic extract of each sample was mixed with 2 mL of 15% Folin–Ciocalteu reagent, 3 mL of sodium carbonate (10%) and incubated at 40°C for 60 min. Finally, a spectrophotometer was used to measure the absorbance at 765 nm. Gallic acid was used as standard.

Agro-morphological traits

Eight agro-morphological traits were measured following the standard evaluation protocols according to Zahidi *et al.*

Table 1: Locality of the collected cowpea landraces from different collection sites

Accessions No.	Accessions name	Province	Location	Altitude	Longitude	Latitude
1	4550Yadoudeh	Amman	Yadoudeh area	906	°E 35 31 48.7	°N 31 30 15.4
2	4543Airporthighway	Amman	Airport highway	835	°E 35 31 54.1	°N 31 30 40.6
3	94Ghernateh	Madaba	Ghernata	819	°E 35 46 53	°N 31 46 39
4	92Ghernatah	Madaba	Ghernata	819	°E 35 46 53	°N 31 46 39
5	913Ghernatah	Madaba	Ghernata	819	°E 35 46 53	°N 31 46 39
6	245Mafiraq	Mafraq	Mafraq	704	°E10 87 36	°N30 58 32
7	244Mafraq	Mafraq	Mafraq	704	°E10 87 36	°N30 58 32
8	95Alhouson	Irbid	Al huson	670	°E35 54 20	°N32 28 17
9	4367Alhouson	Irbid	Al huson	650	°E35 54 33	°N32 29 31.8
10	Ajlun	Ajlun	Abeen	1100	°E47 37 35	°N35 67 32
11	Kufrauan	Irbid	Kufrauan	430	°E58 48 35	°N28 80 32
12	100Batras	Irbid	Bait ras-Maru	521	°E35 52	°N32 36
13	98Hibras	Irbid	Hibras-AL-sa,d	602	°E35 51	°N32 41
14	102Khraj	Irbid	Khraj	476	°E35 53	°N32 40
15	Anbeh	Irbid	Anbh	694	°E27 79 35	°N42 58 32
16	Baytyafa	Irbid	Bayt yafa	617	°E26 07 35	°N05 51 32
17	Albarha	Irbid	Albarha	522	°E59 35 35	°N50 86 32
18	Aqaba	Aqaba	Shalali	136	°E47 13 35	°N53 95 29
19	4552Egypt	EGYPT				

(2013). Seed length (cm), width (cm) and total area (cm^2) were assessed before sowing. Plant height (cm), leaf length (cm), width (cm), internode space (cm) and petiole length (cm) were measured 60 days after germination. IMAGJ software was used to measure the seed length, width and total area for each landrace. Genotypic and phenotypic coefficients of variation were assessed using Burton and Devane's (1953) equations. Genetic advance (GA) was calculated according to Allard (1960):

$$GA = K * \sigma p * h^2 \text{ (b)}$$

Where, K = Selection differential @ 5% selection intensity.

Broad sense Heritability was calculated by $\sigma^2 g/\sigma^2 p$ * 100%.

Where $\sigma^2 g$ is genotypic variance and $\sigma^2 p$ is phenotypic variance.

Statistical analysis

For constructing a dendrogram for molecular and agro-morphological data, NTSYS pc 2.20 Software was used. Data on agro-morphological traits were analyzed using the IBM SPSS statistics 16.

Results

Molecular diversity

Twenty-three ISSR primers were used in this study and produced 259 markers, with an average number of markers for each primer equal to 11.26. UBC835 primer produces the highest number of markers (19 markers), while UBC860 primer produces the lowest number (4 markers). The size of DNA fragments produced by all primers ranged between 200 bp and 1500 bp. Generally, 191 of the markers produced were polymorphic, and the percentage of polymorphism was 87%. The UBC 835 primer recorded the highest primer efficiency (8.48), and the UBC 860 primer recorded the lowest (1.79), averaging 4.35 for all primers used in the study. The discrimination power for the 23 ISSR primers ranged between 2.09 and 8.38. Primer UBC 860 has the lowest value of discrimination power, and UBC 857 has the highest value of discrimination power. The primers UBC815, UBC 899, UBC 864, and UBC 810 could generate 2, 1, 1, and 1 unique bands, respectively.

2667 scored DNA fragments were used to construct genetic similarity between the 19 cowpea landraces used in this study. Table 2 shows the genetic similarity values calculated based on Jaccard's coefficient. The result ranged from 0.46 to 0.86, with an average of 0.67.

The genetic similarity matrix was used to build a dendrogram to show the relativeness between the landraces in the study. The dendrogram illustrates that the samples are clustered in two main groups; the first group includes 4550yadoudeh, 94ghernatah, 92ghernatah, 245mafraq, 95alhouson and 102Khraj, while the second includes 4543Airporthighway, 913ghernatah, 244mafeaq, 4376alhouson, Ajlun, Kufrauan, 100batras, 98Hibras, Anbeh, Baytyafa, Albarha, Aqaba, and 4552egypt, the dendrogram shows that these groups are subdivided into further subgroups (Fig. 1).

Biochemical analyses

This study assessed the phenotypic variation between nineteen cowpea landraces through Biochemical characteristics, as seen in Table 3. The total moisture content of Jordanian cowpea landraces varies from 3.2 to 5.1%, while total phenolics vary from 10.2 to 15.2 mg/100 g. On the other hand, the total protein content of Jordanian cowpea varieties varies from 19 to 26 % (Table 3).

Table 2: Similarity coefficient matrix for pairwise comparison of 19 cowpea landrace

	4550 yadoudeh	4543Airport highway	94 ghernatah	92 ghernatah	913 ghernatah	245 mafraq	244 mafeaq	95 alhouson	4376 alhouson	ajlun	kufrauan	100 batras	98 Hibras	102 Khraj	Anbeh	Bayt yafa	Albarha	Aqaba	4552 egypt
4550 yadoudeh																			
4543Airport highway	0.67																		
94 ghernatah	0.86	0.71																	
92 ghernatah	0.81	0.63	0.80																
913 ghernatah	0.58	0.74	0.66	0.58															
245 mafraq	0.75	0.64	0.79	0.80	0.60														
244 mafeaq	0.63	0.69	0.64	0.63	0.74	0.57													
95 alhouson	0.71	0.55	0.69	0.68	0.46	0.67	0.52												
4376 alhouson	0.57	0.64	0.62	0.60	0.71	0.63	0.72	0.50											
ajlun	0.63	0.70	0.64	0.60	0.63	0.66	0.63	0.59	0.73										
kufrauan	0.61	0.63	0.62	0.62	0.64	0.63	0.73	0.56	0.72	0.76									
100 batras	0.58	0.71	0.61	0.58	0.68	0.60	0.75	0.52	0.74	0.72	0.74								
98 Hibras	0.60	0.71	0.64	0.63	0.71	0.60	0.72	0.57	0.70	0.74	0.71	0.77							
102 Khraj	0.71	0.61	0.73	0.70	0.52	0.70	0.54	0.75	0.54	0.66	0.63	0.59	0.63						
Anbeh	0.63	0.70	0.64	0.61	0.69	0.63	0.68	0.56	0.69	0.75	0.73	0.76	0.76	0.65					
Bayt yafa	0.60	0.69	0.64	0.63	0.72	0.63	0.70	0.54	0.70	0.74	0.74	0.77	0.75	0.65	0.84				
Albarha	0.64	0.67	0.64	0.65	0.67	0.62	0.66	0.59	0.68	0.71	0.72	0.72	0.73	0.64	0.81	0.78			
Aqaba	0.61	0.70	0.60	0.63	0.71	0.66	0.71	0.50	0.73	0.75	0.73	0.79	0.70	0.58	0.79	0.77	0.76		
4552 egypt	0.62	0.69	0.63	0.62	0.71	0.61	0.69	0.52	0.71	0.74	0.75	0.78	0.78	0.61	0.79	0.76	0.79	0.83	

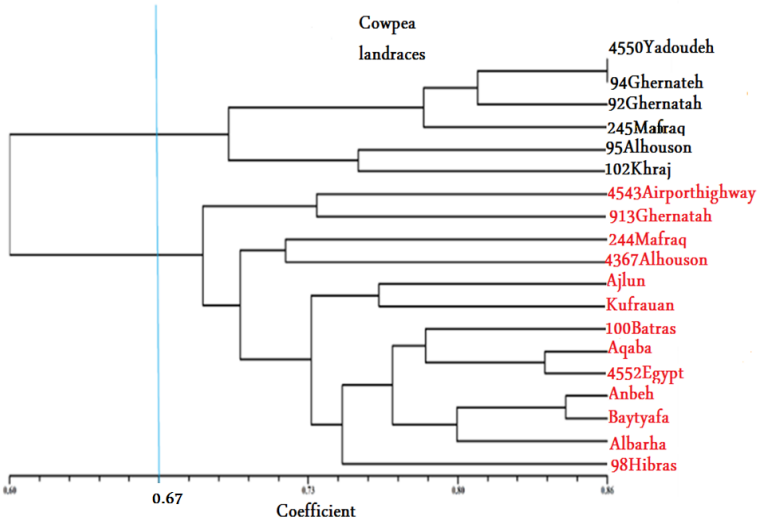

Fig. 1: Dendrogram of nineteen cowpea landraces based on ISSR analysis

Agro-morphological traits and Genetic parameters

Eight agro-morphological traits were measured, including plant height, leaf length, leaf width, petiole length, internode space, seed length, seed width, and seed area. In this study, most phenotypic traits expressed relatively high phenotypic coefficient of variance (PCV). The highest PCV value was recorded for seed width (50.22), while the lowest PCV values were recorded for leaf length (19.55) (Table 4). At the same time, the genotypic coefficient of variation (GCV) ranged from 15.64 to 46.35 for leaf width and seed width, respectively (Table 4). On the other hand, the lowest heritability value was (25.59) for leaf width, whereas seed width and seed length showed high levels of heritability (98.06) and (97.36), respectively (Table 4). Plant length, internode space, leaf length, and seed area showed moderate heritability. Genetic advance (GD) was measured and ranged from 0.28 to 8.52 for seed area and plant height, respectively (Table 4).

The dendrogram was built according to the argo-morphological parameters to study the relatedness between 19 cowpea landraces. The dendrogram illustrates that samples are divided into three major clusters. The first group includes seven landraces as follows: 4450Yadoudeh, Anbeh, 102Khraj, 244Mafraq, 95Alhouson, Ajlun, and 100Batras.

Table 3: Biochemical characterization and Agro-morphological traits of cowpea landraces used in this study

Landrace name	Moisture content (%)	Total phenolics (mg/100 g)	Protein content (%)	Average plant height (cm)	Average length of leaf (cm)	Average width of leaf (cm)	Average petiole length (cm)	Average internode space (cm)	Average seed length (cm)	Average seed width (cm)	Average seed area (cm^2)
4550Yadoudeh	3.2	14.7	22	36.66	7.16	9.00	5.66	3.333	0.742	0.50	0.36
4543Airporthighway	4.7	15.2	21	27.00	6.36	9.33	3.33	2.667	0.888	0.66	0.59
94Ghernateh	3.8	14.1	21	16.66	9.66	8.00	3.00	2.50	1.10	0.66	0.68
92Ghernatah	4.1	14.9	23	22.00	10.33	13.00	3.33	5.83	1.08	0.72	0.71
913Ghernatah	5.1	10.7	24	9.33	6.50	9.50	4.16	6.00	0.96	0.72	0.67
245Mafiraq	4.3	10.8	20	21.16	6.50	8.33	5.66	7.50	0.96	0.53	0.47
244Mafraq	4.7	11.2	24	25.00	8.66	8.00	5.50	3.16	0.93	0.54	0.45
95Alhouson	3.9	14.8	21	20.00	8.66	10.00	6.83	3.16	1.16	0.80	0.78
4367Alhouson	4.3	14.3	20	19.66	7.33	7.00	4.66	3.83	0.80	0.50	0.36
Ajlun	3.8	10.8	24	15.66	7.33	12.33	2.33	3.16	0.81	0.62	0.41
Kufrauan	3.9	10.2	26	20.66	9.00	9.50	4.83	4.66	0.92	0.48	0.40
100Batras	4.5	14.6	23	27.66	10.50	10.16	4.33	4.16	1.20	0.77	0.82
98Hibras	4.2	15.1	19	16.33	7.66	9.33	3.33	3.00	1.05	0.71	0.62
102Khraj	3.9	12.7	22	27.33	8.66	9.67	4.50	3.33	0.89	0.70	0.49
Anbeh	4.9	10.8	24	31.66	8.67	10.67	5.66	3.33	0.88	0.69	0.49
Baytyafa	4.1	11.9	21	16.00	7.66	11.00	2.50	2.83	1.12	0.61	0.58
Albarha	4.4	12.4	23	19.33	6.00	8.33	4.83	2.50	1.12	0.50	0.49
Aqaba	3.8	13.1	21	13.33	7.00	7.50	2.16	2.00	0.91	0.71	0.59
4552Egypt	4.7	10.3	25	19.00	7.00	8.66	3.50	3.00	0.79	0.63	0.45
LSD, *P < 0.05*	0.2	0.4	1.3	1.72	0.46	1.87	0.84	0.42	0.11	0.08	0.06

Table 4: Genetic parameters of agro-morphological traits

	DF	Internode space	Petiole length	Leaf width	Leaf length	Plant height	Seed length	Seed width	Seed area
GV		2.22	0.87	1.21	2.22	36.12	0.185	0.093	0.017
PV		2.93	3.40	4.74	2.93	60.01	0.19	0.103	0.023
PCV%		46.19	45.69	23.7	19.55	36.83	44.94	50.22	27.34
GCV%		40.25	23.09	11.99	18.97	28.57	44.42	46.35	23.41
Broad sense heritability		75.82	24.72	25.59	75.88	62.23	97.36	98.06	74.84
Genetic advance		2.67	0.93	1.07	2.84	8.52	0.85	0.67	0.28

The second group includes six landraces: 94Ghernatah, Albarha, 4376Alhouson, Kufrauan, 913Ghernatah and 245Mafraq. The third group includes the following landraces 4543Airport Highway, 98Hibras, 4552Egypt, 92Ghernatah, Aqaba, and Baytyafa sample (Fig. 2).

Discussion

Landraces are considered a model of an evolutionary process for adaptation and the ability of plants to survive under arid and semi-arid environments (Brown 2000). Landraces provide heterogeneous, adaptive species and genetic resources to meet current challenges for farming in harsh environments (Dwivedi *et al.* 2016). Legumes are the third-largest family of angiosperms. Cowpea is essential as human food and soil fertilization through a symbiotic relationship between nitrogen fixation bacteria and the root of cowpea. It is a significant source of animal feed due to the quality of its leaves (Yahara *et al.* 2013).

Recently, many research papers have revealed the ability of molecular markers to discriminate between genotypes in different species, for example, wheat (Winfield *et al.* 2018) and olive (Mousavi *et al.* 2017). Molecular markers are highly used to evaluate the genetic diversity of plant crops, including landraces. ISSR and other PCR-based molecular markers are potent tools for genetically characterizing cowpea germplasm (Ghalmi *et al.* 2010).

In this study, four primers generate five unique bands, which are valuable for the organization of the germplasm bank and are considered a helpful tool for certification of their plant material if SCAR markers developed from these bands. The genetic similarity matrix shown in Table 2 indicates that the highest genetic similarity was 0.86 between 94Ghernatah and 4550yadoudeh, and the lowest was 0.46 between 95Alhouson and 913Ghernatah landraces. These values are consistent with the geographical location of the collection sites of these landraces. On the other hand, the dendrogram could not divide the samples according to geographical areas. This result could be explained through the scored DNA fragments generated by the ISSR marker, which only covers a small part of the cowpea genome. However, the dendrogram shows three subgroups, including eight landraces clustered according to geographical distribution. The first subgroup includes 4550Yadoudeh, 94Ghernateh, and 92Ghernatah; these samples belong to locations from the Madaba governorate. The second subgroup includes Aqaba and 4552Egypt. Aqaba is the nearest place in Jordan to Egypt. The third subgroup

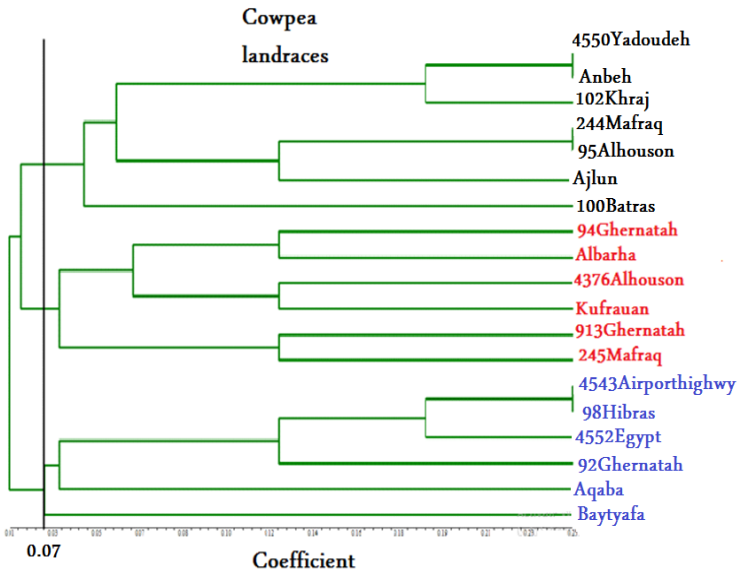

Fig. 2: Dendrogram of nineteen cowpea landraces using morphological and biochemical characteristics

includes samples collected from three places in the Irbid governorate: Anbeh, Baytyafa and Albarha.

Legumes are a rich source of proteins, fibers, carbohydrates, vitamins, and minerals (Kassie *et al.* 2009). Protein legumes are considered a source of crucial amino acids such as lysine. In contrast, they are suffering a decrease in essential sulfur amino acids (methionine and cysteine) and tryptophan. So, it should be consumed with other cereals (Alayachew and Geletu 2017). Our result shows that protein content varied from 19 to 26%, which agrees with Carvalho *et al.* (2012) study. Arif *et al.* (2020) found that genetic and environmental factors highly influenced the variation of protein content and the quality of the seed of dry pea (*Pisum sativum* L.). The major types of proteins were globulins followed by albumins, basic glutelins, acid glutelins and prolamins, respectively (Vasconcelos *et al.* 2010).

The number of landraces used in this study was relatively small, but they are considered a representative sample of all existing cowpea landraces in Jordan. As expected, distinct morphological characteristics were found between these landraces. In this study, nineteen cowpea landraces showed a significant variation in all morphological parameters. Agro-morphological variation has been reported by Ghalmi *et al.* (2010) for different characters in cowpea landraces. Also, Egbadzor *et al.* (2014) studied 118 cowpea genotypes collected from Ghana, Nigeria and the United States of America. The results showed highly significant differences among 16 morphological traits that were studied. In the present study, five growth parameters, including plant height, leaf length, width, internode space, and petiole length, showed high variation; similarly, Alam and Hossain (2008) reported marked variation for traits like plant height, petiole length, leaf length, leaf width and internode space of 50 okra (*Abelmoschus esculentus* L.) landraces. The variation in agro-morphological characteristics could be due to environmental factors, like annual precipitation, temperature, elevation, or the hybridization between landraces. Elibox and Umaharan (2012) studied sixteen morphological parameters of 82 Anthurium accessions grown in the Caribbean. They found significant variation between these accessions and claimed it is due to climate and other environmental factors.

Our results showed that the phenotypic coefficient variance (PCV) ranged from 19.55 to 50.22 for leaf length and seed width, respectively. The genotypic coefficient of variation (GCV) ranged from 11.99 to 46.35 for leaf width and seed width, respectively. The PCV value was higher than GCV for all traits, which reflects the higher environmental effect on the expression of traits in this experiment. Our result agrees with Al-Tabbal and Al-Fraihat (2012), who found a vast morphological variation in 86 barley genotypes and three checks. They explained that this variation is due to wide seasonal variability, low rainfall and poor soil moisture. Another study investigated a total of 576 genotypes of sweet potato; they found that the significant source of variation is due to environmental factors but not genetic background (Wera *et al.* 2015).

Broad sense heritability (h^2) is a genetic parameter that measures the genetic contribution to phenotypic variance. In this study, h^2 ranged from 24.72 for petiole length to 98.06 for seed width. This considerable variation could be due to the genetic variation among landraces rather than the environmental variation. Similarly, Akhtar *et al.* (2011) evaluated the genetic variability of RICE (*Oryza sativa* L.) heritability of several growth parameters, including plant height and number of grains panicle. They found broad sense heritability, genetic solid association, and a direct effect on

these parameters. Genetic advance (GA) under selection also showed significant variation from 0.28 for seed area to 8.52 for plant height. These results are supported by other researchers who conducted a study to estimate genetic variability and heritability in bread wheat (*Triticum aestivum* L.). They found that genetic advances vary highly among the measured parameters (Ali *et al.* 2008).

The morphology dendrogram broadly clustered Cowpea landraces into three major groups, which refers to a high level of morphological diversity. Jawarneh *et al.* (2013) studied the genetic diversity of three Quercus species in Jordan from 25 natural populations. The morphological character analysis of *Q. calliprinos* was grouped into three sub-clusters according to the geographical distribution: the southern, middle, and northern. However, these results disagree with our results, where no association between morphological characteristics and geographical distribution was observed in the present study. Our result could be explained by Smith and Donoghue (2008), who explained the spread of some plant lineages around the Northern Hemisphere after they adapted to cold tolerance. They have similar morphological traits, such as Gentianella, Halenia and Lupinus, but different in molecular genetic makeup.

Depending on the species, molecular marker results could be used as a supplement, a complement, and/or an alternative for distinctness testing based on morphological characters. Based on the results of this study, the usefulness of molecular marker results could be considered as a supplement to the morphological analysis. The association between molecular markers and agro-morphological traits is dependent on the genetic groups that are investigated; two scenarios may occur if molecular results show that two genotypes are close together: (1) even though they are close at the molecular marker level, the genotypes could be from two distinct origins. Thus, they should show agro-morphological variation in some (if not most) characteristics. (2) Genotypes are close at the molecular marker level and share the exact origin. This will result in similar (if not identical) agro-morphological traits. Accordingly, both scenarios appear complementary and functional in investigating the variation (Bar-Hen *et al.* 1995).

Differences in clustering patterns were observed not only between agro-morphological and molecular markers but also between different types of molecular markers. Ferrada-Noli (1997) assessed the genetic relationships between 9 barley cultivars using data from restriction fragment length polymorphisms (RFLPs) or random amplified polymorphic DNAs (RAPD). They found a completely different clustering pattern between RFLP and RAPD. Roldan-Ruiz *et al.* (2001) used a group of ryegrasses (*Lolium perenne* L.) varieties to assess the morphological characterization and molecular markers (AFLP and STS) association in describing varieties relationships. They found inconsistent relationships between morphological and molecular analysis. In another study, the result of an association between morphological classifications of the Demospongiae G4 clade with the molecular analysis of the large subunit ribosomal RNA (LSU rRNA) sequences showed a massive conflict between the current morphological classification and the LSR rRNA analysis (Morrow *et al.* 2012).

Conclusion

Our results showed that landraces were separated into two main groups based on ISSR analysis. On the other hand, the agro-morphological group studied landraces into three clusters. Neither genetic nor agro-morphological/biochemical dendrograms were related to geographical distribution.

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Author Contributions

WAK designed and supervised the research. IS and MA conducted experiments and data collection. MB, BAS and MJ conducted statistical analysis. WAK, MB and IS writing the original manuscript. FA, MYJ, and MA review and editing. BAS, MYJ, MJ and FA Figures illustrations. All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

Authors declare no conflict of interest.

Data Availability

Data will be available upon request.

Ethics Approval

Not applicable to this study.

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