Asian Journal of Biotechnology and Genetic Engineering

Volume 6, Issue 1, Page 46-57, 2023; Article no.AJBGE.99526

Assessment of Genetic Diversity in Some Landraces of Common Beans Using Inter-Simple Sequence Repeat (ISSR)

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Author's contribution

The sole author designed, analysed, inteprted and prepared the manuscript.

Article Information

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/99526

Original Research Article

Received: 12/03/2023
Accepted: 15/05/2023
Published: 24/05/2023

ABSTRACT

Aims: Use molecular tools to characterize six common beans genotypes, which were collected from distinct geographical origins in Egypt. This is to explore the existence of genetic variability that may help to generate strategies for conservation and potential utilization in future beans breeding programs.
Study Design: Completely randomized.
Place and Duration of Study: This investigation took place from June to December 2022 at the laboratory of the vegetable seed of Sabahya Horticulture Research Station, Alexandria Governorate, Agricultural Research Center, Egypt.
Methodology: Inter-Simple Sequence Repeat (ISSR) marker was employed to evaluate the efficiency in diversity analysis of common beans genotypes.
Results: There were genetical diversity among the six studied genotypes of common beans according to the used four markers of ISSR.
Conclusion: It is important to collect local varieties and determine their genetic diversity in order to

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protect genetic resources and use them in breeding studies. The records obtained in this study make it possible to establish the basis to generate use and conservation strategies for the evaluated common beans genotypes. There is genetic variability among beans genotypes from different geographical locations. ISSR markers were effective in genetically distinguishing the evaluated materials despite their expected high homozygosity due to the autogamous nature of this crop.

Keywords: Common beans; ISSR marker; genetic diversity; landraces.

1. INTRODUCTION

Common beans (*Phaseolus vulgaris* L.) belongs to family Fabaceae is an annual, diploid (2n=22) species. Genetic variability in domesticated common beans is low. This is maybe due to the fact that most cultivars within each gene pool were developed from a relatively narrow genetic base [1].

Among the tools used to estimate genetic diversity in a set of genotypes, molecular markers enable direct estimation of genetic diversity at the DNA level, reducing the interference of environmental variation, and they are not influenced by the environment [2].

DNA molecular markers include inter simple sequence repeat (ISSR) markers, which are widely used in genetic diversity studies because they are universal and highly polymorphic, require single primers, have a low cost of development and have high reproducibility of results [3].

The most impact of climate change over previous and the next decades will surely be on agriculture and food security. In fact, these changes have been seen to cause a decrease in the variability of those genetic loci (alleles of a gene) controlling physical and phenotypic responses to changing climate. Therefore, genetic variation holds the key to the ability of populations and species to persist over evolutionary period of time through changing environments. Nonetheless, the current genetic composition of a crop species influences how well its members will adapt to future physical and biotic environments [4].

Therefore, the main objective of this study is to use molecular tools to characterize six common beans genotypes, which were collected from distinct geographical origins in Egypt. This is to explore the existence of genetic variability that may help to generate strategies for conservation and potential utilization in future beans breeding programs.

2. MATERIALS AND METHODS

This investigation took place from June to December 2022 at the laboratory of the vegetable seed of Sabahya Horticulture Research Station, Alexandria Governorate, Agricultural Research Center, Egypt.

2.1 Plant Material

Six genotypes of common beans were collected from Egypt representing six geographical different locations [5]. These genotypes included one registered cultivar namely Nebraska (Registered cultivar at Horticulture Research Institute) and non-registered cultivar called Dandara collected from Sohag governorate. The rest of genotypes were collected from the governorates Alexandria, Kafr El-Sheikh, Aswan, and Assiut.

2.2 ISSR Analysis

Genomic DNA was extracted from seeds of the six common beans genotypes by using DNA extraction kits (Easy Pure Plant Genomic DNA Kit). DNA samples were stored at -20◦C. DNA quality was checked by electrophoresis in a mini gel. In the present study, ISSR marker was employed to evaluate the efficiency in diversity analysis of common beans genotypes according to Doðan [5-6]. The sequences of the used primers are shown in Table 1. PCR reactions were performed in 25µl total volume, using 2µl from diluted DNA, 2µl of each primer for the amplification reaction, 12µl master mix (Taq Ready Mix PCR Kit from the fast gene) and 9µl ddH_2O (sterile water) for all reactions. The tubes were capped and placed in a thermocycler and the cycling was started immediately. Amplification protocol was carried out using PCR cycler 600 programmed for initial denaturation step at 94◦C for 15 min, followed by 35 cycles each at 94◦C for 30 sec, annealing at 57◦C for 30 sec and extension at 72◦C for 8min.

The products of ISSR based PCR analyses were detected using agarose gel electrophoresis

(2.5% in 1X TBE buffer) stained with ethidium bromide (0.3µl). PCR products were visualized on U.V. light; photographed and analyzed using Total Lab Quant soft wear program [7].

2.3 Statistical Procedures

Molecular data and cluster analysis, data were scored for computer analysis on the basis of the presence of the amplified products for each primer. If a product was present in a genotype, it was designated as “1”, if absent, it was designated as “0”, after excluding the unreproducible bands. Pair-wise comparisons of genotype, based on the presence or absence of unique and shared polymorphic products, were used to determine similarity coefficients, according to Jaccard [6]. DNA fragment size was estimated by comparison with a 1.5-kbp DNA ladder Ready to use from Gene Direx. The similarity coefficients were then used to construct dendrograms, using the Unweighted Pair Group Method with Arithmetic Averages (UPGMA) employing the SAHN (Sequential, Agglomerative, Hierarchical, and Nested clustering) from Past program version 4.03.

3. RESULTS AND DISCUSSION

Assessment of genetic variability of germplasm is a first step, named as pre-breeding, for improvement and development of superior cultivars. Four primers for ISSR technique were screened for their ability to amplify the genomic DNA of the six studied common beans genotypes. Since ISSR primers are dominant markers, data were analyzed based on the comparison of the amplified fragments using gel documentation for each primer. If a fragment was present in a sample, it was designated as "1", if absent, it was designated as "0". If a fragment was present or absent in the genotype then absent or present in the others, it was called a unique species-specific marker, but if a fragment was absent and present in more than one genotype, it was called polymorphic finally if the fragments were present in all genotypes, it was called monomorphic.

A total of 82 ISSR fragments were amplified with the four used primers ranged from 17 (primer 3 and4) to 27 (primer 2), zero of them were common fragments (monomorphic), 50 of them showed to be polymorphic and other 32 showed to be unique fragments (Tables 2 - 5 and Fig. 1).

The percentage of polymorphic (ranged from 42.9 in primer NO.1to 76.5 in primer NO.3) and unique bands (ranged from 23.5 in primer NO.3 to 57.1 in primer NO.1) obtained for the six genotypes are shown in Table (6). Genetic polymorphisms determine the diversity of individuals [8-11].

Cabral [5] compared markers that are codominant and dominant they found that codominant higher percentage of polymorphism more than dominant markers polymorphisms in the common beans. The results are similar to those found by other authors, although there is variation in the polymorphism percentage due to the discard of weak-intensity bands that were not analyzed. Generally codominant markers are more informative than the dominant markers.

According to our results it can be concluded that the ISSR markers revealed high level of polymorphism in common beans genotypes. Thus, ISSR markers can be used in determination of genetic relationships, since it is easy to apply and have high level of reproducibility [6,7].

The number of specific amplified fragment (AF) and Amplified fragments obtained for the six genotypes are exhibited in Tables (7 and 8). The ISSR polymorphic and unique banding pattern analysis has been successfully used for molecular characterization and detection of genetic variability of genotypes in various crop plants.

There are two main ways of analyzing the resulting distance (or similarity) matrix, namely, principal coordinate analysis (PCA) and dendrogram (or clustering, tree diagram). Dendrogram (or tree diagram), that is, grouping of samples together in clusters that are more genetically similar to each other than to samples in other clusters. Different algorithms were used for clustering, but some of the more widely used ones include unweighted pair group method with arithmetic averages (UPGMA), neighbour-joining method, and Ward`s method [4]. The molecular data can be scored in presence/absence matrices manually or with specific software. However, because these techniques are based on the incorporation of genomic elements in the primer sets or else target specific regions in the genome, biases affecting the evaluation process can occur. Although many recently developed targeting methods detect large numbers of polymorphisms. These mainly affect the analysis of the banding patterns produced, largely depending on the nature of the methods and whether they generate dominant or codominant markers [12–14].

UPGMA dendrogram demonstrate the distribution of the studied genotypes with all the 4 polymorphic ISSR reactions. Cluster analysis, according to DNA- ISSR analysis divided the 6 studied genotypes into groups as shown in Table (9) and Fig. (2). Among these clusters, there was a mono-genotypic cluster and the other included between 2 to 5 genotypes with a number of sub-clusters. Genotypes swapped among different clusters. These results may be due to the diversity at the molecular level, which may not reflect the diversity at the morphological or physiological level, as described by [8]. Another possible reason for this variation in clustering might be the environmental influence and genotype-environment interaction [15].

Table 1. Sequences and annealing temperature of the ISSR primers used in the study

Primer's code	Sequence (5´-3´)
14A	*(CT)8TG*
49A	*(CA)6AG*
HB-9	*(CTC)3(TCT)2TGC*
HB-12	*(CAC)3GC*

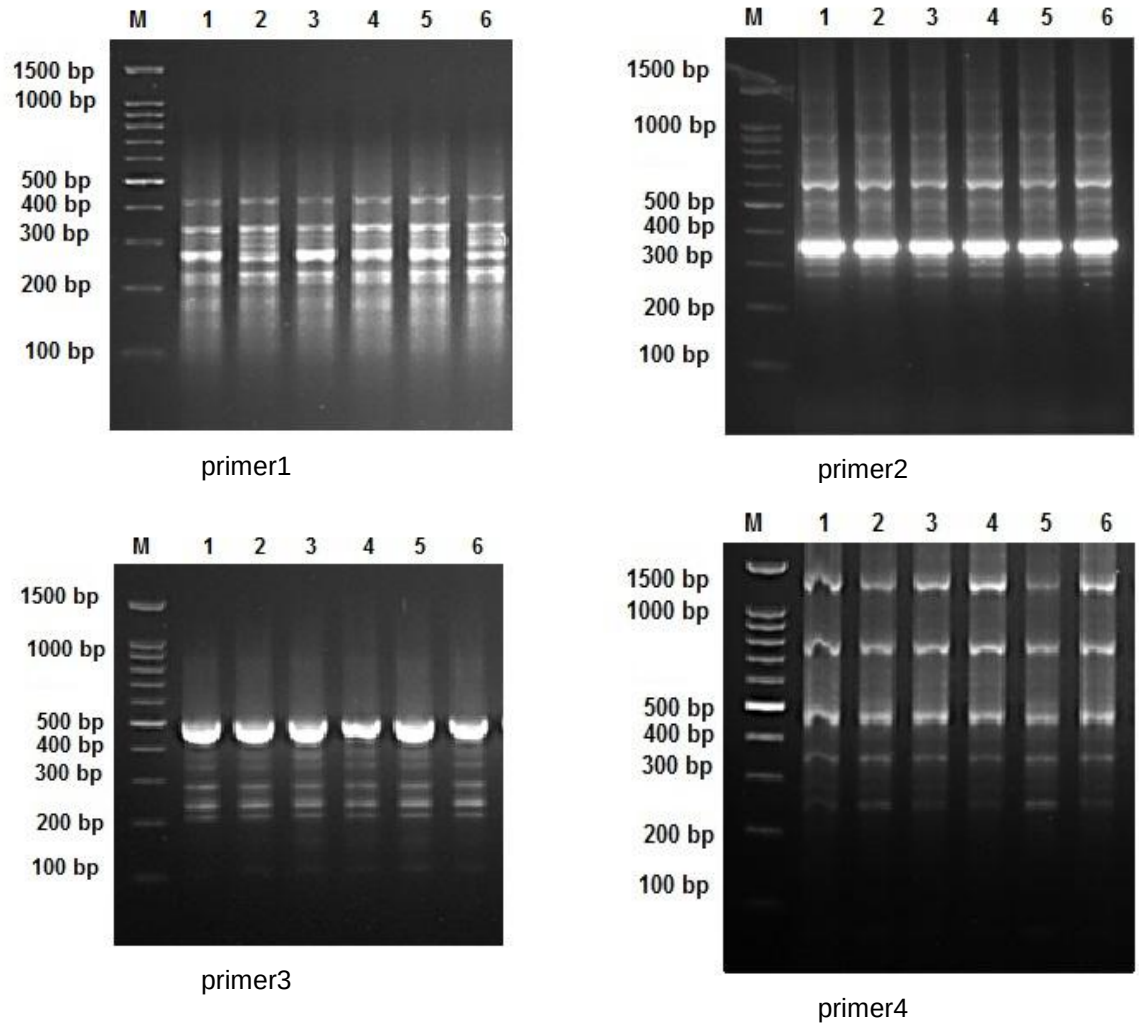

Fig. 1. ISSR banding patterns in the six genotypes accessions generated using 4 primers. (1, 2,3,4,5 and 6 for Nebraska, Assiut, Dandara, Kafr El-sheikh, Aswan and Alexandria, respectively)

Table 2. Amplified DNA fragments (AF) obtained for the six genotypes using first ISSR primer

NO.	MW#	RF#	Nebraska	Assiut	Dandara	Kafr El-sheikh	Aswan	Alexandria	Polymorphism
1	415	0.41	0	0	0	0	0	1	Unique
2	408	0.49	0	0	1	0	1	0	Polymorphic
3	402	0.55	0	1	0	1	0	0	Polymorphic
4	390	0.61	1	0	0	0	0	0	Unique
5	325	0.69	0	0	0	0	0	1	Unique
6	321	0.40	0	0	0	1	1	0	Polymorphic
7	316	0.48	0	1	1	0	0	0	Polymorphic
8	307	0.57	1	0	0	0	0	0	Unique
9	278	0.61	0	0	0	0	0	1	Unique
10	259	0.40	0	0	0	1	0	0	Unique
11	256	0.68	1	0	1	0	1	1	Polymorphic
12	246	0.47	0	1	0	0	0	0	Unique
13	221	0.55	0	0	0	0	1	1	Polymorphic
14	219	0.74	0	1	1	1	0	0	Polymorphic
15	216	0.60	1	0	0	0	0	0	Unique
16	187	0.68	1	0	0	0	0	1	Polymorphic
17	182	0.39	0	0	0	0	1	0	Unique
18	180	0.47	0	0	1	0	0	0	Unique
19	178	0.52	0	1	0	1	0	0	Polymorphic
20	163	0.67	0	0	0	0	0	1	Unique
21	160	0.73	0	0	0	1	0	0	Unique
Detectable fragments			5	5	5	6	5	7	33

 # MW: molecular weight; RF: retardation factor

Table 3. Amplified DNA fragments (AF) obtained for the six genotypes using second ISSR primer

NO.	MW[#]	RF[#]	Nebraska	Assiut	Dandara	Kafr El-sheikh	Aswan	Alexandria	Polymorphism
1	1430	0.13	0	1	1	0	0	0	Polymorphic
2	1405	0.24	0	0	0	0	1	1	Polymorphic
3	1381	0.38	0	0	0	1	0	0	Polymorphic
4	1201	0.43	0	0	0	1	0	0	Unique
5	1180	0.56	0	0	1	0	0	1	Polymorphic
6	959	0.59	1	0	0	0	1	1	Polymorphic
7	942	0.64	0	0	1	1	0	0	Polymorphic
8	926	0.12	1	0	0	0	1	0	Polymorphic
9	729	0.37	0	0	0	0	1	1	Polymorphic
10	716	0.43	0	1	0	1	0	0	Polymorphic
11	595	0.61	0	1	0	0	0	1	Polymorphic
12	585	0.17	1	0	1	0	1	0	Polymorphic
13	504	0.24	0	0	0	0	1	1	Polymorphic
14	496	0.48	1	0	1	0	0	0	Polymorphic
15	487	0.60	0	1	0	1	0	0	Polymorphic
16	442	0.32	0	0	0	0	0	1	Unique
17	428	0.42	0	0	1	1	0	0	Polymorphic
18	336	0.63	0	0	0	0	0	1	Unique
19	331	0.12	1	1	1	1	1	0	Polymorphic
20	297	0.17	0	0	0	1	0	0	Unique
21	292	0.23	0	0	1	0	0	0	Unique
22	283	0.31	0	1	0	0	1	0	Polymorphic
23	279	0.65	1	0	0	0	0	1	Polymorphic
24	262	0.47	0	0	0	1	0	0	Unique
25	258	0.55	1	0	1	0	0	0	Polymorphic
26	255	0.61	0	0	0	0	0	1	Unique
27	251	0.64	0	0	0	0	1	0	Unique
Detectable fragments			7	6	9	9	9	10	50

 # MW: molecular weight; RF: retardation factor

Table 4. Amplified DNA fragments (AF) obtained for the six genotypes using third ISSR primer

NO.	MW#	RF#	Nebraska	Assiut	Dandara	Kafr El-sheikh	Aswan	Alexandria	Polymorphism
1	455	0.44	1	0	1	1	0	0	Polymorphic
2	446	0.53	0	1	0	1	1	1	Polymorphic
3	362	0.52	0	0	0	0	1	1	Polymorphic
4	354	0.57	0	1	0	0	0	0	Unique
5	348	0.63	1	0	1	0	0	0	Polymorphic
6	325	0.79	0	0	0	0	1	1	Polymorphic
7	317	0.44	0	1	1	1	0	0	Polymorphic
8	308	0.50	1	0	0	0	0	0	Unique
9	266	0.58	0	0	0	0	1	0	Unique
10	264	0.42	0	1	0	1	0	0	Polymorphic
11	256	0.66	1	0	0	0	0	1	Polymorphic
12	226	0.80	0	0	0	1	0	0	Unique
13	220	0.49	0	1	1	0	1	1	Polymorphic
14	214	0.52	1	0	1	1	1	0	Polymorphic
15	202	0.62	0	1	1	0	1	1	Polymorphic
16	196	0.78	1	0	1	1	0	0	Polymorphic
17	127	0.57	0	1	1	1	1	1	Polymorphic
Detectable fragments			6	7	8	8	8	7	44

 # MW: molecular weight; RF: retardation factor

Table 5. Amplified DNA fragments (AF) obtained for the six genotypes using fourth ISSR primer

NO.	MW#	RF#	Nebraska	Assiut	Dandara	Kafr El-sheikh	Aswan	Alexandria	Polymorphism
1	1328	0.09	1	0	0	0	0	0	Unique
2	1287	0.23	0	1	0	1	1	1	Polymorphic
3	1270	0.39	0	0	1	0	0	0	Unique
4	1120	0.51	0	1	0	1	0	0	Polymorphic
5	798	0.60	1	0	0	0	0	0	Unique
6	774	0.10	0	1	0	1	1	1	Polymorphic
7	766	0.24	0	0	1	0	0	0	Unique
8	646	0.40	0	0	0	0	1	0	Unique
9	582	0.50	0	0	0	1	0	0	Unique
10	459	0.60	1	0	0	0	0	0	Unique
11	446	0.10	0	1	0	1	0	1	Polymorphic
12	442	0.25	0	0	1	0	0	0	Unique
13	326	0.50	1	0	1	1	0	1	Polymorphic
14	322	0.14	0	0	0	0	1	1	Polymorphic
15	313	0.32	1	0	1	0	0	0	Polymorphic
16	235	0.29	1	1	0	0	1	1	Polymorphic
17	232	0.50	0	1	1	0	0	0	Polymorphic
Detectable fragments			6	6	6	6	5	6	35

 # MW: molecular weight; RF: retardation factor

Table 6. ISSR pattern of the six genotypes using 4 primers

ISSR primer	MB#	PB#	UB#	TAP#	P%#	U%#
1	0	9	12	21	42.9	57.1
2	0	19	8	27	70.4	29.6
3	0	13	4	17	76.5	23.5
4	0	9	8	17	52.9	47.1
Total	0	50	32	82	61.0	39.0
Average	0	20	12.8	32.8	60.7	39.3

 # MB: monomorphic bands; PB: polymorphic bands; UB: Unique bands, TAP: total amplified pattern; P%: percentage of polymorphic bands; U%: percentage of Unique bands

Table 7. Amplified specific DNA fragments (AF) obtained for six genotypes using ISSR primers

Primers	Genotypes						Total
	Nebraska	Assiut	Dandara	Kafr El-sheikh	Aswan	Alexandria	
1	3	1	1	2	1	4	12
2	0	0	1	3	1	3	8
3	1	1	0	1	1	0	4
4	3	0	3	1	1	0	8
Total	7	2	5	7	4	7	32

Table 8. Amplified DNA fragments (AF) obtained for the six genotypes using ISSR primers

Primers	Genotypes						Total
	Nebraska	Assiut	Dandara	Kafr El-sheikh	Aswan	Alexandria	
1	5	5	5	6	5	7	33
2	7	6	9	9	9	10	50
3	6	7	8	8	8	7	44
4	6	6	6	6	5	6	35
Total	24	24	28	29	27	30	162

Table 9. Grouping of genotypes on the basis of morphological and molecular data by using PAST4.03 program

P1	Genotypes	P2	Genotypes	P3	Genotypes	P4	Genotypes	Four primers	Genotypes
A	Nebraska and Dandara	A	Nebraska[#]	A	Alexandria, Aswan and Assiut	A	Nebraska and Dandara	A	Nebraska and Dandara
B	Kafr El-sheikh, Assiut, Alexandria and Aswan	B	Alexandria, Aswan , Kafr El-sheikh, Dandara and Assiut	A1	Alexandria and Aswan	B	Kafr El-sheikh, Assiut, Alexandria and Aswan	B	Kafr El-sheikh, Assiut, Alexandria and Aswan
B1	, Kafr El-sheikh and Assiut	B1	Alexandria and Aswan	A2	Assiut[#]	B1	Kafr El-sheikh and Assiut	B1	Kafr El-sheikh and Assiut
B2	Alexandria and Aswan	B2	Dandara and Assiut	B	Nebraska , Kafr El-sheikh and Dandara	B2	Alexandria and Aswan	B2	Alexandria and Aswan
				B1	Nebraska				
				B2	Kafr El-sheikh and Dandara				

 # Mono-genotypic clusters

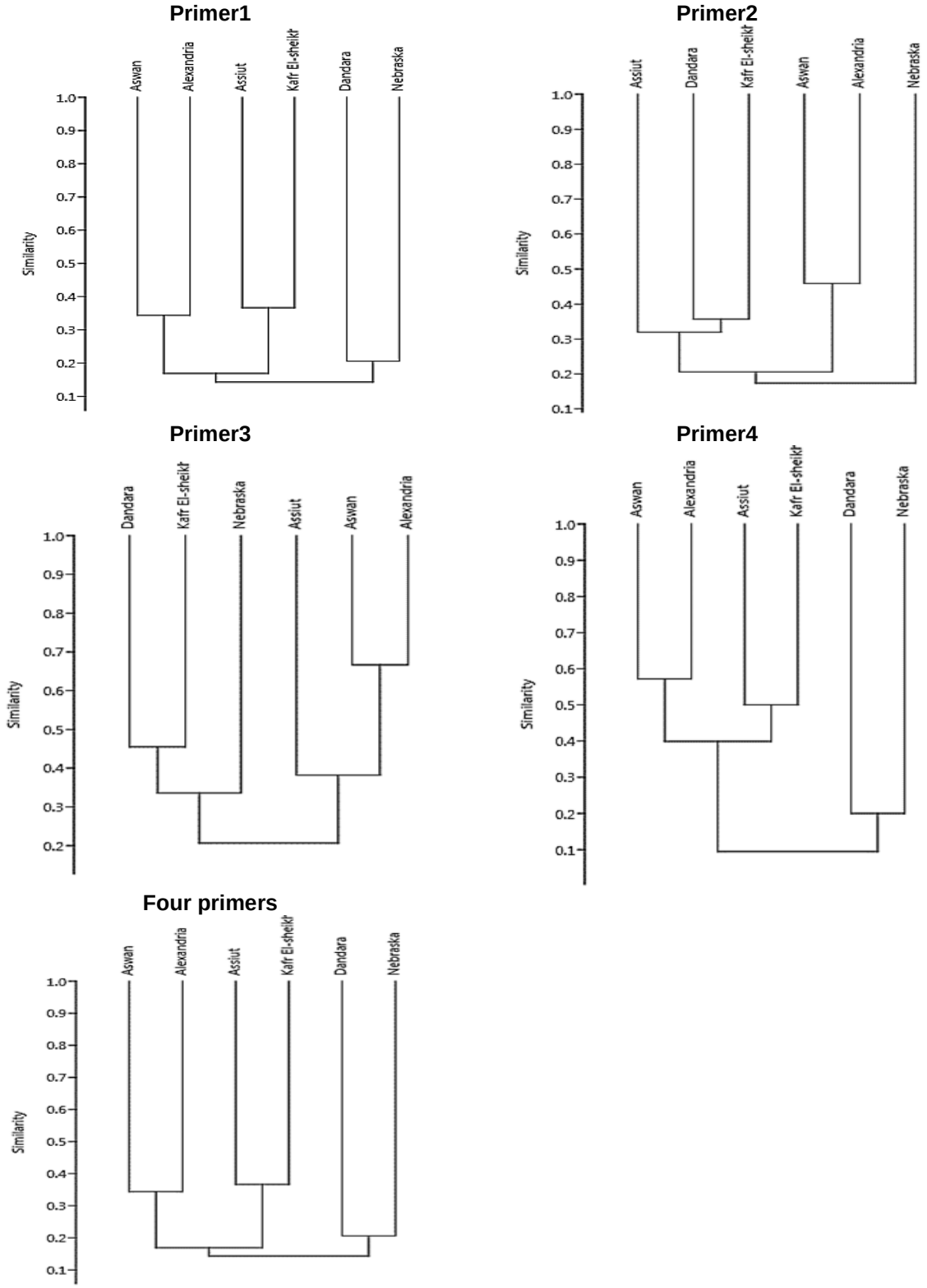

Fig. 2. Cluster analysis using UPGMA method depicting genetic similarity (Jaccards coefficient) between six genotypes derived from band sharing data of ISSR

4. CONCLUSIONS

Assessment of genetic variability of the germplasm is the first step, termed pre breeding, for the improvement and development of superior cultivars. In the present study, genotypes were collected from six geographical isolated locations one of them was registered cultivar, were evaluated at the molecular level. It is important to collect local varieties and determine their genetic diversity in order to protect genetic resources and use them in breeding studies. The records obtained in this study make it possible to establish the basis to generate use and conservation strategies for the evaluated common beans genotypes. There is genetic variability among beans genotypes from different geographical locations. ISSR markers were effective in genetically distinguishing the evaluated materials despite their expected high homozygosity due to the autogamous nature of this crop.

COMPETING INTERESTS

Author has declared that no competing interests exist.

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