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Diversity and Population Structure Analysis of Faba Bean (*Vicia faba* L.) Accessions Using SSR Markers

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ABSTRACT

The awareness and conception of the genetic diversity in faba bean (*Vicia faba*) accessions are important for the enforcement of degree addressed to their usages and conservations. The aim of this work was to estimate the genetic diversity and population structure in Iraqi faba bean using SSR markers for utilization in crossing and variety development. To assess genetic variation and population structure among faba bean accessions, 25 microsatellite loci were exerted. The analysis of diversity indices in the set of faba bean accessions examined here showed that the microsatellites were informative for genotype characterization. In total, 72 polymorphic alleles were exposed to an average of 2.88 per locus and three unique alleles were detected. The average of *PIC*, gene diversity, marker index, resolving power and Shannon diversity was 0.513, 0.569, 1.671, 2.173 and 0.830, respectively. The patterns detected in the dendrogram and PCA divided 19 accessions into five distinct clusters with different levels of sub-grouping within the cluster. High-level genetic differentiation within a population or group (83%) was significantly greater than that among groups or populations (17%), as planned by Analysis Of Molecular Variance (AMOVA). The model of clustering, based on the analysis of STRUCTURE software, identified four groups genetically dispersed. These findings have additional importance in faba bean breeding as well as maintenance programs.

Keywords: Genetic variation indices, Genetic structure, Legume, Microsatellites.

INTRODUCTION

Faba bean (*Vicia faba* L.), also called broad bean, and horse bean originated in the Mediterranean-West Asia region (Tanno and Willcox, 2006). The today faba bean has an important role in human food and animals feed worldwide because of its contents of protein (27-34%), starch (45%) and essential vitamins (Mejri *et al.*, 2012). Faba bean is a diploid species (2*n*= 2*x*= 12 chromosomes). The size of genomic DNA approximately is 13.000 Mb (Ellwood *et al.*, 2008). In Iraq, faba bean is one of the fundamental legume plants grown mostly under rain-fed conditions. The unreliability of the yield is largely due to the lack of adequate genetic tool and feasible resistance/tolerance to the prevailing diseases (*Ascochyta*), pests, and drought. Hence, it is crucial to evolve new accessions that are able to tolerate the biotic and abiotic stresses accountable for low productivity in Iraq. The display of modern genetic tools with improved agronomic traits and increased genetic variation leads to the expansion of high-yielding accessions (Hwang *et al.*, 2008). In this circumstance, a convenient description of the genetic divergence at the morphology and molecular levels in the accessible local faba bean accessions resource is indispensable for additional improvement of yield and stress

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tolerance. The agro-morphological traits are unsuitable to the development of gene pools because these traits are affected by environmental factors and stage of plant development, or they demonstrate merely limited diversity (Terzopoulos and Bebeli, 2008). In faba bean, several molecular techniques have been profitably employed to appraise the genetic relationship, such as isozyme (Mancini *et al.*, 1989; Tahir, 2015), Restriction Fragment Length Polymorphism (RFLP) (Torres *et al.*, 1993), Inter-Simple Sequence Repeat (ISSR) (Salazar-Laureles, 2015), Amplified Fragment Length Polymorphism (AFLP) (Zong *et al.*, 2009), Randomly Amplified Polymorphic DNA (RAPD) methods (Tair, 2015), target region amplification polymorphism (TRAP) (Kwon *et al.*, 2010), Single Nucleotide Polymorphism (SNP) (Kaur *et al.*, 2014) and Simple Sequence Repeats (SSRs) (Kaur *et al.*, 2012). SSR markers are often applied in plant diversity and population structure because they are easy, PCR-based, highly polymorphic, and can be used to differ between closely related accessions. To develop *V. faba* accessions, an introductory description, estimation, and comparison of the genetic variation among regional and ICARDA accessions could be helpful for the imposition of this genetic variability in breeding programs. The aim of this study was to evaluate the genotypic diversity, relatedness and population structure among local *V. faba* resources used in Iraq and the cultivated ICARDA accessions based on SSR markers.

MATERIALS AND METHODS

Genotype Collection

A total of 19 faba bean (*Vicia faba* L.) accessions, including eight local and eleven ICARDA genotypes, was investigated in the present work (Table 1), these accessions were gathered from the Agriculture Research, Ministry of Agriculture, Sulaimani, Kurdistan region, Iraq.

DNA Extraction and Electrophoresis

Genomic DNA was isolated from faba bean fresh leaves by the CetylTrimethylAmmonium Bromide (CTAB) procedure (Doyle, 1990). The

Table 1. Name of faba bean accessions examined in the study and their origin.

No.	Code	Accessions	Origin
1	G1	Zaffaron	Iraq
2	G2	Viola	Iraq
3	G3	Tolera	Iraq
4	G4	Baby green	Iraq
5	G5	Local check	Iraq
6	G6	Seher	Iraq
7	G7	Yieldiz	Iraq
8	G8	Cirilla	Iraq
9	G9	FLIP12-031	ICARDA-Lebanon
10	G10	FLIP12-032	ICARDA-Lebanon
11	G11	FLIP12-034	ICARDA-Lebanon
12	G12	FLIP12-037	ICARDA-Lebanon
13	G13	FLIP12-041	ICARDA-Lebanon
14	G14	FLIP12-044	ICARDA-Lebanon
15	G15	FLIP12-046	ICARDA-Lebanon
16	G16	FLIP12-047	ICARDA-Lebanon
17	G17	FLIP12-056	ICARDA-Lebanon
18	G18	FLIP12-063	ICARDA-Lebanon
19	G19	Giza-3	ICARDA-Lebanon

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quality and concentration of the extracted DNA were determined by 1% (w/v) agarose gels quantified using a Bio-Rad gel imaging system. The extracted genomic DNA was diluted to 70 ng μL^{-1} and stored at −20°C.

Molecular Marker (SSR) Assay

Out of 30 markers (Zeid *et al.*, 2009, Yang *et al.*, 2012, Suresh *et al.*, 2013), 25 primers were successfully amplified and utilized for diversity and structure studies (Table 2). PCR reaction was conducted in a 20 µL reaction containing 1X PCR buffer, 250 mM dNTPs, 0.30 µL of primer, 3 mM $MgCl_2$, 1 µL Taq polymerase and 70 ng template DNA. PCR amplification was conducted using Applied Biosystems Thermocycler following the PCR protocol: Initial denaturation at 94°C for 8 minutes, 38 cycles at 94°C for 1 minute, T°C (T°C depending upon the primer pair) for 1 minute, 72°C for 2 minutes and a final extension step at 72°C for 7 minutes. Amplified products were resolved on 2.5% agarose gels at 80 V in 1X TBE buffer, and fragment sizes were determined by 100-bp DNA ladder (Invitrogen, USA). The size of bands was scored using the GelAnalyzer software.

Scoring and Statistical Data Analysis

The scorable bands were coded manually as either present (1) or absent (0). Scored data were applied to the calculation of Jaccard's similarity coefficient using XLSTAT 11 software. The Jaccard's coefficient was converted to a dissimilarity matrix to create dendrogram using the Unweighted Pair-Group Method with Arithmetic averages (UPGMA) by using XLSTAT 11 software. Polymorphism Information Content (PIC), gene diversity and major of allele frequency were computed by using PowerMarker version 3.25 software. To determine the relationship between different accessions, the Principal Component Analysis (PCA) was conducted by XLSTAT 11 software. GenAlEx version 6.5 software was also used to estimate the molecular variance among and within populations (Peakall *et al.*, 2012). Marker Index (MI) and Resolving Power (RP) are measured according to Powell *et al.* (1996) and Prevost and Wilkinson (1999). To population structure, a model analysis was fulfilled to infer genetic structure and to clarify the number of sub-populations using the STRUCTURE software version 2.3.4 (Pritchard *et al.*, 2000, Evanno *et al.*, 2005). The ancestry and allele frequency models applied in this work, were admixture model and allele frequencies correlated, respectively. The number of supposed populations (K) was set from 1 to 10, and the analysis was repeated 6 times. For each run, the burn-in and MCMC were fixed to 50,000 each and iteration was deposited in 6. The run with the highest likelihood was used to set accessions into sub-populations.

RESULTS AND DISCUSSION

Allelic Variation in Faba Bean Accessions Using SSR Markers

Out of 30 primers, 25 primers revealed amplified fragments. The lack of amplification of some microsatellites primer was due to an annealing failure of primer. This annealing defeat can be due to differences in the base sequences of the loci between faba bean accessions. For each SSR primer, total number of amplified bands, number of polymorphic bands, percentage of polymorphic bands, number of monomorphic bands, size of amplified bands, number of unique bands, Polymorphism Information Content (PIC), gene diversity, frequency of the major allele, marker index, resolving power and Shannon's diversity index were measured (Table 3). The SSR markers proved to be reproducible and informative polymorphisms. The results obtained are presented in Tables 3. The total numbers of amplified and polymorphic bands were recorded according to clarity and their

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Table 2. Primer names, sequences, motif and annealing temperature of SSR markers used in this work.

No	Primer name	Sequence: 5'- 3'	Motif	Annealing temperature (°C)
1	CAAS1	*F: AGTCAGGGGGTCGATTTTTC* *R: TCTTGCGCAGTTTTGACATC*	(AAAGGG)7	55
2	CAAS2	*F: TACAAAAGCTCTGGGGCCTA* *R: CCAATTCCTCTGGGCAACT*	(GAA)9	56
3	CAAS4	*F: ATTGCAAGTCCTGAGGCAAG* *R: ATAATGGCGCCACAAAGTGT*	(CA)11	55
4	CAAS5	*F: TACATCAGTCCCGCAAATCA* *R: CCATGTAGCCGATTCCACTT*	(ACA)15	55
5	CAAS6	*F: TGCAAAGTAATTCCGAAACAA* *R: CGCACATGAATTGGGGTAAT*	(A)10	56
6	CAAS7	*F: GACCCAAGCCTTCACCACTA* *R: TGTGTGGGATCCATTTTGAA*	(A)10	60
7	CAAS8	*F: AATTTGTTCAGCATCTCGGG* *R: CTGGTTGGTTCCTGGTGAGT*	(AAC)14	56
8	CAAS9	*F: GTGATGCTTTGCCTGTGCTA* *R: ATGGACGTTTGTAGGTGGGA*	(AAC)9	56
9	CAAS11	*F: TCCCGCTATTCTTGCTCTGT* *R: GCTCAAAAATGCTTGTCTTTCA*	(ACA)10	55
10	CAAS12	*F: GAGGAGGATCCCACAATGAA* *R: GCCAAAAGAGCCATGGTAGA*	(TGT)9	56
11	CAAS14	*F: CCGTAGATCTCAAAAACCATGA* *R: GGAGGAAGGAAGCTCGAATC*	(AAC)6	60
12	CAAS15	*F: AACCAACATCAATGGCATCA* *R: TCTTTTCCTTTTTCCTCTTCCA*	(AAC)8	60
13	CAAS16	*F: TCAAATTTCCCTTTGCAAAAAT* *R: GACCAAGGTCAACCACCTTT*	(CA)7	55
14	GBSSR-VF-38	*F: ACCATTTGGCCTGTTCCT* *R: CGCTACCCAAATGCTGAA*	(GTG)6	58
15	GBSSR-VF-164	*F: ACCATTTGGCCTGTTCCT* *R: CAAGGAGGGTTGTTTACGA*	(CAC)6	60
16	GBSSR-VF-311	*F: GGCCTTTCAACAAGAGGG* *R: ACCATTTGGCCTGTTCCT*	(GTG)6	58
17	VfG1	*F: TTTCAGCAAACTAGAACCAATC* *R: GGCATTCAGTTTTTACCTTGTA*	(AG)15	50
18	VfG3	*F: TTCTTTGGTCCTCTCTCTATC* *R: GCACTGTTGTTGCTGATACAA*	(AG)7	50
19	VfG4	*F: AAGGGGAGGGCATAACAGAA* *R: AATCCGCAAGGGTCTTCTTT*	(AG)5	50
20	VfG9	*F: GGTTTTGAATAGAAATGCAA* *R: AAGATGTGTCAATATTGTTTT*	(AG)16	50
21	VfG10	*F: ACCAAAACGCGCACTTATCA* *R: AAGAGAGAGAAGAGAGCTTC*	(AG)5	50
22	VfG11	*F: GCAAAAGGAGAGCAAGGGAA* *R: CGAAAGAGGGGGACATTTTGT*	(AG)8	50
23	VfG13	*F: GGTTGGGATCTTTTAGGTTGAA* *R: TGGCCTTATATCCGTCCAAT*	(AG)10	50
24	VfG19	*F: AGCGATGGTGCTCATGCTTA* *R: TCTCTCACGGAATCACATCTTT*	(AG)9	50
25	VfG27	*F: CCCAAAAAGAGACGAACTGTAT* *R: AGGGTTCATACGTTTGGCTT*	(AG)9	50

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Table 3. Genetic diversity indices from SSR primer for screening 19 accessions of faba bean.[a]

Primer name	TNAB [a]	NPB [b]	NPB% [c]	NMB [d]	Size (bp) [e]	NUB [f]	PIC [g]	GD [h]	MAF [i]	MI [j]	RP [k]	H [l]
CAAS1	4	2	50.000	2	180-290	0	0.536	0.615	0.474	1.072	1.263	0.645
CAAS2	6	6	100.000	0	195-780	0	0.705	0.742	0.368	4.227	3.263	1.822
CAAS4	2	2	100.000	0	170-200	0	0.357	0.465	0.632	0.714	2.000	0.658
CAAS5	4	4	100.000	0	140-295	0	0.813	0.831	0.316	3.254	2.737	1.358
CAAS6	3	2	66.667	1	140-330	0	0.544	0.609	0.526	1.087	1.684	0.628
CAAS7	4	4	100.000	0	200-350	0	0.661	0.709	0.421	2.642	4.000	1.316
CAAS8	2	2	100.000	0	150-185	0	0.374	0.499	0.526	0.749	2.000	0.692
CAAS9	4	3	75.000	1	200-690	0	0.688	0.731	0.368	2.064	2.526	0.826
CAAS11	2	2	100.000	0	200-240	0	0.524	0.598	0.526	1.049	1.684	0.702
CAAS12	3	3	100.000	0	50-280	0	0.504	0.571	0.579	1.512	2.526	0.661
CAAS14	3	3	100.000	0	230-300	0	0.492	0.576	0.526	1.475	2.211	0.929
CAAS15	2	2	100.000	0	120-140	0	0.359	0.410	0.737	0.717	1.895	0.553
CAAS16	4	4	100.000	0	285-330	0	0.416	0.438	0.737	1.666	3.895	0.724
GBSSR-VF-38	2	2	100.000	0	220-245	0	0.257	0.277	0.842	0.515	1.789	0.300
GBSSR-VF-164	2	2	100.000	0	200-230	0	0.359	0.410	0.737	0.717	1.895	0.553
GBSSR-VF-311	2	1	50.000	1	210-390	0	0.367	0.484	0.588	0.367	0.737	0.368
VfG1	2	1	50.000	1	220-260	0	0.186	0.208	0.882	0.186	0.211	0.237
VfG3	3	3	100.000	0	160-220	1	0.555	0.626	0.474	1.665	1.895	0.861
VfG4	6	5	83.333	1	180-610	0	0.799	0.820	0.316	3.995	2.842	1.712
VfG9	3	3	100.000	0	180-420	0	0.525	0.560	0.632	1.576	2.211	0.752
VfG10	2	1	50.000	1	200-230	0	0.178	0.198	0.889	0.178	0.211	0.237
VfG11	4	2	50.000	2	200-700	0	0.524	0.598	0.526	1.049	1.263	0.645
VfG13	4	4	100.000	0	200-550	2	0.509	0.593	0.474	2.035	2.316	0.986
VfG19	6	6	100.000	0	190-800	0	0.823	0.842	0.211	4.939	4.737	1.737
VfG27	3	3	100.000	0	185-400	0	0.774	0.803	0.263	2.322	2.526	0.849
Min	2	1	50.000	0		0	0.178	0.198	0.211	0.178	0.211	0.237
Max	6	6	100.000	2		2	0.823	0.842	0.889	4.939	4.737	1.822
Average	3.280	2.880	87.000	0.400		0.120	0.513	0.569	0.543	1.671	2.173	0.830
Total	82	72		10								

[a] Total Number of Amplified Bands, [b] Number of Polymorphic Bands, [c] Percentage of Polymorphic Bands, [d] Number of Monomorphic Bands, [e] Size of Amplified Bands, [f] Number of Unique Bands, [g] Polymorphism Information Content, [h] Gene Diversity, [i] Frequency of the Major Allele, [j] Marker Index, [k] Resolving Power and [l] Shannon diversity index.

molecular weight concerning the DNA Ladder. A total of 82 reproducible alleles was detected with a molecular weight ranging from 50 to 800 bp along the different faba bean accessions. Twenty-five SSR primers amplified 72 polymorphic bands. The number of alleles varied from 1 (GBSSR-VF-311 and VfG1and VfG10) to 6 (CASS2 and VfG19) with a mean of 2.880 by locus. Among 25 primers used, only VfG1 (1 band) and VfG11 (2 bands) showed unique band. A singular band is a band amplified in an accession, but not in the rest of accessions. It is useful in the marker-assisted selection. The considerable number of mean detectable and polymorphic fragments might be due to the sequence length variations of the primers used in this study. The difference in the number of alleles amplified by various primers is affected by primer sequence and fewer number of annealing locations in the genome. Comparing our results with previous studies, we found that the number of alleles per locus in our results (3.28) was inferior to that obtained by Rebaa *et al*. (2017), which showed 6.62 alleles per locus. Abid *et al.* (2015) obtained 101 polymorphic bands, and a range from 2 to 10 alleles per primer, with a mean of 5.94 alleles per marker on the screening of forty-six faba bean accessions through the 17 SSR loci.

According to Zeid *et al.* (2009), 53 polymorphic bands were detected during the discriminating of 10 faba bean accessions by using 73 SSR markers. The number of amplified fragments is comparable to those described for faba bean cultivars in other countries such as Korea. Certainly, the number of amplified bands for the SSR loci publicized by Suresh *et al*. (2013) ranged from 2 to 15. In addition, the mean number of alleles (3.28) was similar compared with the mean number of alleles per locus (3.37) for 53 faba bean accessions from Portugal, Spain, and Morocco with 28 SSR primers. (Oliveira *et al.*, 2016). The variation in the number of alleles per locus between our results and previous researches, can be explained by the difference in the used genotypes and primers.

The markers with many alleles are considered as highly informative and powerful markers. The power of marker can be quantitatively measured by several methods of statistics called the Polymorphism Information Content (PIC), Marker Index (MI), Resolving Power (RP), and Shannon's diversity index (H), which were used to assess the information of the SSR primers in this work. Primers with a diversity of statistics index values of zero or less than zero should not be used for analysis, due to lesser degree of information for gene diversity. The greatest value of the diversity index intend to a higher degree of polymorphism of the SSR markers and, therefore, helped to select the best SSR loci in the genetic divergence analysis. *PIC* value varied from 0.178 (VfG10) to 0.823 (VfG19) with a mean of 0.513, gene diversity ranged from 0.198 (VfG10) to 0.842 (VfG19) with an average of 0.569, the frequency of the major allele aligned from 0.211 (VfG19) to 0.889 (VfG10) with a mean 0.543 across all accessions (Table 3). The average values of marker index, resolving power, and Shannon index (H) was 1.671, 2.173 and 0.830, respectively. Primer VfG19 was very informative due to its high ability to detect divergence among faba bean accessions. The values of diversity indices designate the discriminatory power of the used SSR primer to distinguish the accessions. The value of genetic diversity of this work is superior to that reported by Gol *et al.* (2016), who studied the genetic diversity in faba bean accessions from Turkey.

Several previous studies demonstrated the power of SSR in the discrimination of faba bean. The *PIC* values discovered are similar to those reported for faba bean cultivars in other countries. Indeed, the *PIC* values for the SSR loci exhibited by Suresh *et al*. (2013) ranged from 0.16 to 0.88, by Zeid *et al.* (2009) varied from 0.16 to 0.72, by Gong *et al.*, 2011 reached from 0.064 to 0.428, by Abid *et al.* (2015) varied from 0.38 to 0.84,

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and by Oliveira *et al.* (2016) extended from 0.071 to 0.662.

Clustering and Principal Component Analyses (PCA)

Clustering and main component analyses were applied for assessing the linkage between faba bean accessions. Jaccard similarity coefficients were between 0.314 (G9 *vs.* G11) to 0.891 (G6 *vs.* G15), signifying an agreeable representation of the genetic distance among the nineteen faba bean accessions. Our result is in accordance with the reporting of Kumari *et al.* (2013) that indicated the existence of a high degree of genetic variation, which ranged from 0.11 to 0.73 among the twenty-eight accessions. In the dendrogram constructed by Jaccard similarity coefficients using unweighted pair-group method (UPGMA), all 19 accessions clustered into five clusters (A, B, C, D, and E) with a mean similarity of 0.410 (Figure 1). Cluster A included G6, G7, and G8. Cluster B comprised G17. Cluster C included G1, G2, G3, G4, and G5. Cluster D consisted of G18, and the remaining seven accessions belonged to cluster E. Within the group, two, four, and five subgroups were detected in groups A, C, and E, respectively. Interestingly, all ICARDA faba bean accessions were observed in group A, B, D, and E. Indeed, accessions 17 and 18 from ICARDA were quite distant from the other accessions, suggesting a high level of genetic diversity.

To better clarify the association between the accessions, PCA was carried out using the similarity data set (Figure 2). The biplot graph of the two first Components (PC1 and PC2) displayed 19.29 and 13.04% of the total distinction, showing a cumulative variance of 32.33%. A high degree of overlapping appeared in the scatter plot that arranged accessions into distinctive clusters (Figure 2). PCA classified nineteen faba bean accessions into five dissimilar clusters. PCA output was congruent with the results generated by UPGMA clustering (Figure 1). In clustering and PCA, G17 and G18 were farthest from the remaining accessions, and

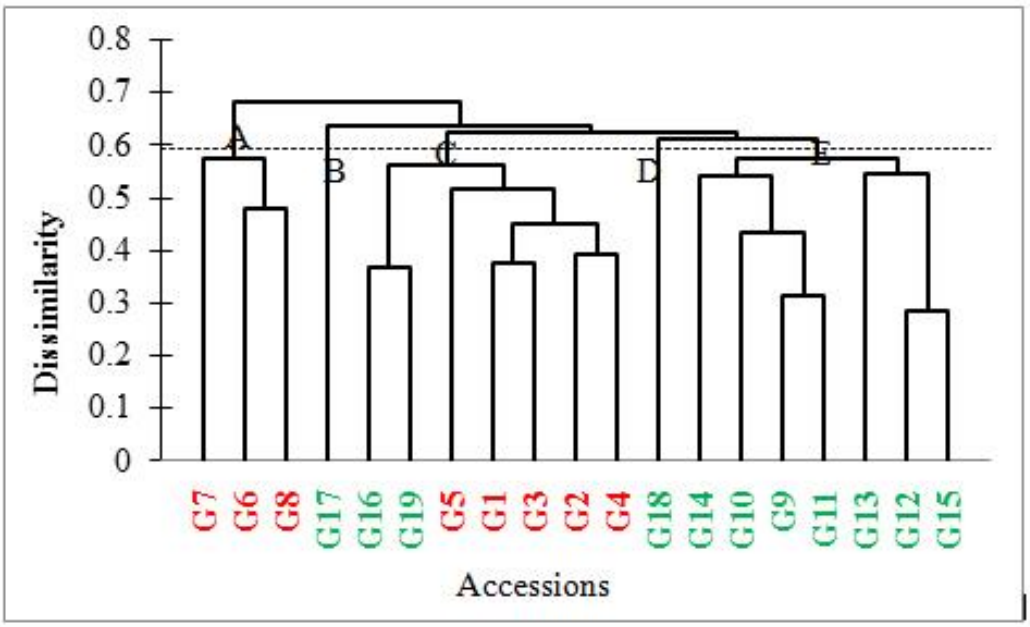

Figure 1. Cluster tree created by UPGMA method based on 25 SSR markers among 19 accessions of faba bean. Accessions: G1: Zaffaron, G2: Viola, G3: Tolera, G4: Baby green, G5: Local check, G6: Seher, G7: Yieldiz, G8: Cirilla, G9: FLIP12-031, G10: FLIP12-032, G11: FLIP12-034, G12: FLIP12-037, G13: FLIP12-041, G14: FLIP12-044, G15: FLIP12-046, G16: FLIP12-047, G17: FLIP12-056, G18: FLIP12-063, G19: Giza-3.

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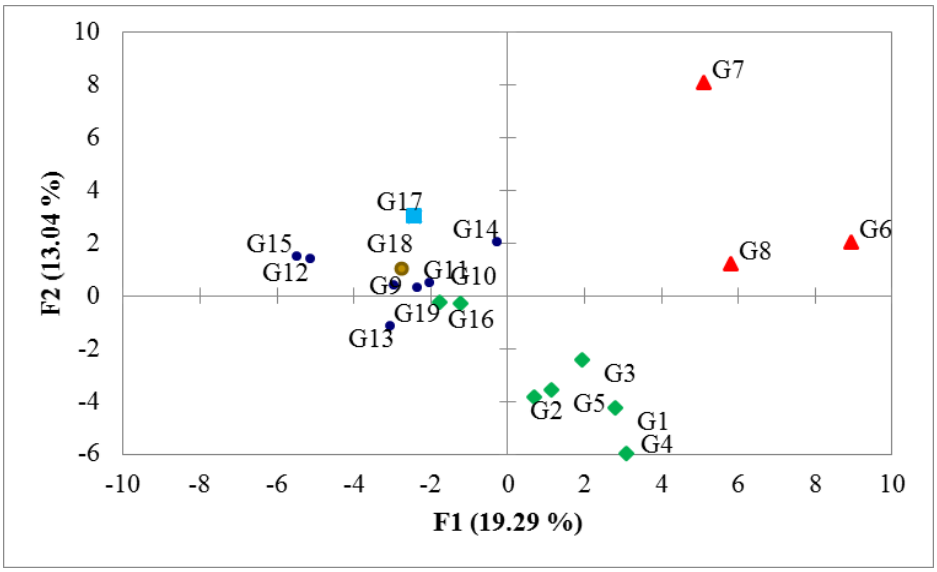

Figure 2. PCA plot of the first and second components among 19 accessions based on genetic variation in 25 SSR marker. Different clusters are denoted by different shapes. Accessions symbols are defined under Figure 1.

as such, they showed the lowest similarity coefficient values. Moreover, PCA analysis showed that all local faba bean accessions were clustered separately from the accessions of another origin. The regional accessions (red color) belonging to the A and C groups (as inferred by UPGMA clustering) were distributed to the right of the plot. The ICARDA accessions (green color) of four groups (B, C, D, and E) were placed in the left section of the plot.

The analysis of AMOVA revealed significant levels of genetic variation (P< 0.01) among populations (local and ICARDA) and individuals within populations. AMOVA of the two sub-populations demonstrated that most of the variation occurred in the intra-sub-populations (83% of the total variation), and only 17% could be ascribed to differences between sub-populations (Table 4). This result also confirmed the existing of great variation within populations. Oliveira *et al.* (2016) analyzed the variance among cultivated types of faba bean through SSR loci showing the highest proportion of genetic distance (36%) within accessions and not among different accessions (10%). An AMOVA analysis through SSR marker was utilized for 35 Northern Africa, Eastern Africa, and Near East, faba bean germplasms detecting a low proportion of genetic difference within populations (75.4%) compared to our results (El-Esawi, 2017).

Genetic Sructure in Faba Bean Accessions

In order to collect information about population structure in faba bean accessions

Table 4. Analysis of molecular variance (AMOVA) of the two sub-populations of faba bean accessions.

Source	*df*	*SS*	*MS*	*Est var*	%	*P* value
Among Pops	1	40.626	40.626	2.905 **	17%	0.001
Within Pops	17	233.216	13.719	13.719**	83%	0.001
Total	18	273.842		16.623	100%	

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depending on allele frequencies and not on provenance or taxonomy; we used the method, STRUCTURE analysis. Analysis of population structure was carried out to detect the number of populations that may be created from 19 accessions using 25 SSR markers. In this analysis, the real *K* value with the highest value of *K* for the 19 individuals was *K*= 6 followed by peaks at *K*= 2 (Figure 3). By the method of Evanno *et al* (2005), all the analyzed accessions were split into four groups or sub-populations (Figure 4). Here, four sub-populations with the strong mixing of the accessions were acquired as shown in Figure 4. Each individual was characterized by a single color line, and each cluster was denoted by a color. The greater area of a color that an individual expected, the greater the opportunity that the individual belonged to the matching group. Groups 1 (green line) and 2 (yellow line) included all local accessions, while groups 3 (violet line) and 4 (blue line) consisted of ICARDA accessions. Accessions in the admixture class may have partial ancestry in more than one background. They possibly had an intricate history involving intercrossing or

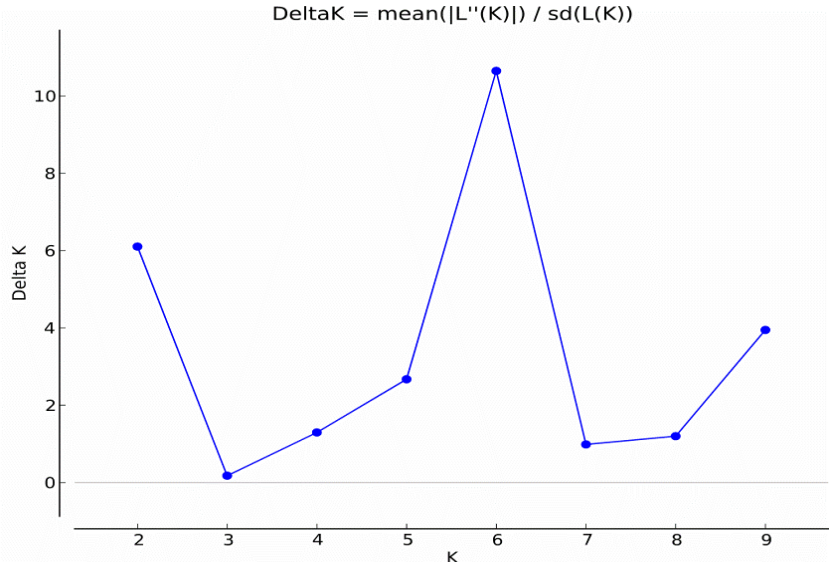

Figure 3. Determining the optimal value of *K* by the (ΔK) procedure described by Evanno *et al.* (2005).

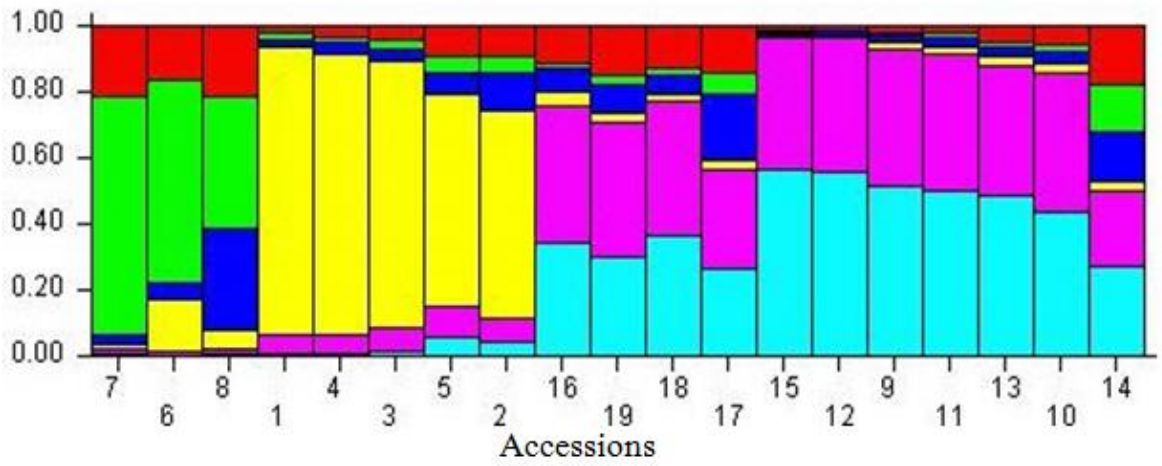

Figure 4. Nineteen accessions of faba bean clustered into different sub-populations by STRUCTURE software. Accessions are coordinated as per estimated membership coefficients (q) in *K*= 6 clusters. Accessions symbols are defined under Figure 1.

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feasibly resulting from the gene flow between taxa. Relative low percentages of pure accessions in both clusters indicate high admixture and natural interbreeding of accessions. Cross-pollination of faba bean is expedited by insect pollinators like honeybees, bumblebees and diverse solitary bees. This part of the result demonstrated a lofty gene flow between local and ICARDA groups. Since the places of a collection of samples are linked to each other, it is possibly a reason for having a blend between two populations.

CONCLUSIONS

SSR marker methods have provided beneficial information in terms of detection of the level of polymorphism and characterization in faba bean accessions. In this work, there was manifest variation between the local and ICARDA accessions, as evident in the dendrogram and PCA plot. Furthermore, we detected the level of variation in genetic structure present in the accessions distributed across different regions of Iraq. The results of the current study can be supportive in scheduling the exploitation patterns and management of faba bean existing in north Iraq. Moreover, the results can be useful to the breeders to select effectively the parents leading to progenies with high differentiation among them. It similarly allows the finest arrangement of faba bean gene pool management and more effective sampling of the obtainable accessions resources. Hence, further investigations in these accessions can possibly add novel alleles to biotic or abiotic stress tolerances, which are important for faba bean breeding.

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تجزیه تحلیل تنوع و ساختار جمعیتی لوبیای فابا (*Vicia faba* L.) با استفاده از نشانگر های SSR

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چکیده

آگاهی و درک مفهوم تنوع ژنتیکی نمونه های ثبت شده لوبیای فابا (*Vicia faba*) برای اعمال درجه بندی در مصرف و حفاظت آنها مهم است. هدف این پژوهش تخمین تنوع ژنتیکی و ساختار جمعیتی لوبیای فابا درعراق با استفاده از نشانگر های SSR برای کاربرد در تلاقی و تولید ارقام بود. به منظور ارزیابی تغییرات ژنتیکی و ساختار جمعیتی در میان نمونه های ثبت شده لوبیای فابا، ۲۵ مکان ژنی ریزماهواره اعمال شد. تجزیه شاخص های تنوع در مجموعه لوبیا فابا های بررسی شده در این مطالعه نشان داد که ریز ماهواره ها برای مشخص کردن ژنوتیپ ها مفید و آموزنده بودند. در کل، ۷۲ آلل پلیمورفیک به طور میانگین در معرض ۲/۸۸ مکان ژنی قرار داده شد و ۳ آلل منحصر به فرد تشخیص داده شد. میانگین PIC، تنوع ژنتیکی، شاخص نشانگر، resolving power، و تنوع شانون، به ترتیب برابر بود با ۰/۵۱۳، ۰/۵۶۹، ۱/۶۷۱، ۲/۱۷۳، و ۰/۸۳. الگوهای شناسایی شده در دندروگرام و تجزیه به مولفه های اصلی(PCA)، ۱۹ نمونه ثبت شده را به ۵ خوشه متمایز دسته بندی کرد که هر خوشه زیر-گروه بندی متفاوتی داشت. همان گونه که طبق تجزیه واریانس ملکولی (AMOVA) برنامه ریزی شده بود، تمایز ژنتیکی در سطح بالا در درون یک جامعه یا گروه (۸۳٪) به طور معناداری بیشتر از تمایز بین گروه ها یا جامعه ها (۱۷٪) بود. مدل خوشه بندی بر مبنای تجزیه نرم افزار STRUCTURE، چهار گروه را که از نظر ژنتیکی پراکنده بودند شناسایی کرد.این یافته ها در برنامه های بهنژادی و نگهداری لوبیای فابا مورد استفاده دارند.

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