



Detection of Genetic Diversity of *Vicia faba* L Using ISSR DNA Markers

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ABSTRACT

Background

Legumes, scientifically classified as Fabaceae or Leguminosae, constitute the third most extensive family of flowering plants, surpassed only by (Orchidaceae) and asters (Asteraceae). The genus *Vicia faba* L., which is temperate and herbaceous in nature, belongs to the legume tribe Vicieae of the Papilionoideae. Legumes and cereals are the two most important flowering plants used in agriculture. Legumes are useful as human and animal food, and as soil-improving components of agricultural and agroforestry systems.

Materials and methods

The present study involved the extraction of DNA from the foliage of the *Vicia faba* L plant, followed by its amplification using three primers for the purpose of conducting ISSR analysis by polymerase chain reaction (PCR).

Results

This process resulted in the generation of a total of 30 DNA fragments, which were then able to be assessed across all genotypes. The aggregate count of polymorphic markers and the mean percentage of polymorphism were recorded as 43.33%.

Conclusion

The findings of our study indicate a substantial degree of genetic diversity within the fenugreek treatments. This discovery also suggests that the ISSR technique provides more comprehensive information for assessing genetic diversity and connections among populations of *Vicia faba* L.

Keywords: *Vicia faba* L., Genetic polymorphisms, ISSR PCR



INTRODUCTION

Faba bean (*Vicia faba L.*), known by several names such as wide bean, field bean, tick bean, windsor bean, and horse bean, holds the distinction of being one of the earliest cultivated crops and is highly regarded for its significant protein content, making it a useful source of nutrition for both people and animals [1,2]. According to Siddique et al. [3], *Vicia faba L.* grain of varying sizes serves different purposes, with large-seeded grain mostly utilized as food, medium-sized grain serving as both food and feed, and small-sized grain predominantly used as feed.

The classification of the plant genus *Vicia faba L.* (with a chromosome count of $2n = 10, 12, 14, 24, 28$; and a basic chromosome number of $x = 5, 6, 7$) places it into the tribe Viciaeae, which is a part of the larger family Fabaceae. The genus consists of herbaceous species that are either annual or perennial, and they are found in temperate climates across Europe, Asia, and North and South America [4,5]. This particular genus exhibits significant variation in genome size, with a range of several fold, as seen by the measurements of 3.85 pg in *Vicia monantha* and 27.07 pg in *Vicia faba* [6,7].

The taxonomic classification of the genus has traditionally relied on morph typological taxonomy, where subgenera and sections are defined based on different diagnostic features selected by various researchers [8]. In their study, Maxted et al. [9] classified the genus into two subgenera, namely *Vicilla* and *Vicia*, as previously proposed by Kupicha [4,10]. The two subgenera can be differentiated based on several characteristics, including stipule nectary, peduncle length, style type, keel form, legume, and canavanine [10]. According to Maxted's [9] classification, the subgenus *Vicia faba* was categorized into nine sections, nine series, 38 species, 14 subspecies, and 22 varieties. The northern Mediterranean region is considered the primary center of variety and a potential genesis for subgenus *Vicia faba*. The geographical area under consideration comprises Iraq, Iran, the southwestern republics that were once part of the Soviet Union, Syria, and Turkey [5].

The limited access to data pertaining to the genetic diversity and intra-specific relatedness in *Vicia faba L.* has posed limitations on the potential for genetic improvement, as well as the effective conservation and administration of its germplasm resources. Researchers in the field of applied plant breeding have shown considerable interest in the utilization of RAPD and ISSR markers. The study conducted by Karp et al. [11] determined that RAPD is a straightforward and effective DNA-based technique, particularly due to its ability to operate without requiring sequence information. The utilization of this genetic instrument enables the estimation of genetic diversity and genetic analysis as well [12]. In addition, the utilization of RAPD approaches offers several benefits due to their little DNA requirement and capacity to detect a substantial number of polymorphisms [13].

The Inter-simple sequence repeat (ISSR) PCR method is a cost-effective and efficient genotyping technology. Inter-Simple Sequence Repeats (ISSRs) refer to specific DNA segments that typically range in size from 100 to 3000 base pairs (bp).

These fragments are situated inside the genomic areas that are positioned between neighboring microsatellite regions, with the microsatellites being oriented in different directions. One of the primary benefits of ISSRs is the elimination of the requirement for sequence data in the design of primers. The aforementioned marker exhibits predominantly dominant characteristics. This method is commonly employed to assess the genetic relatedness between populations. The combination of various parents in hybrid development can lead to the attainment of genetically superior hybrids with a higher magnitude of heterosis. The evaluation of genetic diversity before the development of hybrids can facilitate the more effective utilization of heterosis. The utilization of molecular markers for evaluating genetic variation presents a compelling alternative to traditional methods of diversity analysis, offering potential benefits for biodiversity management and conservation [14].

The objective of the current study was to examine the genetic diversity among various populations of *Vicia faba L* using ISSR DNA markers.

METHODOLOGY

Sample collection

Samples were obtained from various places in Iraq throughout the period spanning from September 2021 to December 2021.

Genotypic identification

DNA Extraction

The DNA of *Vicia faba L*, a plant species, was obtained and purified using the "wizbio" extraction and clearing kit manufactured in South Korea.

Primers

The ISSR primers were acquired from Bioneer and IDTDNA (USA). The DNA of the *Vicia faba L* plant was subjected to testing using particular primers for the ISSR-PCR technique. (table1).

Table (1): Primer Sequences used in this study

primer name	Sequence 5-----3	Reference
ISSR13	5- GAGAGAGAGAGAGAY -3	Isikber <i>et al</i> [22]
UBC-878	5- GGA TGG ATG GAT GGAT -3	
UBC-880	5- GGA TGG GGT GGG GTG-3	

Polymerase Chain Reaction

The final composition of the reaction volumes, which amounts to 25 μ l, comprises 1 μ l each of the forward and reverse primers, 12.5 μ l of the green Master, and 3 μ l of an additional component. The genomic deoxyribonucleic acid (DNA) and reaction rate were quantified and standardized to a volume of 25 microliters (μ l). The amplification process was conducted using a thermo cycler manufactured by Eppendorf. The thermo cycler was designed to run for a period of 5 minutes. The experimental conditions involved subjecting the sample to a temperature of 94°C for a total of 35 cycles. Each cycle consisted of a 1-minute step at 94°C, followed by a 1-minute step

at 55°C, and finally a 2-minute step at 72°C. The process concluded with a final extension step lasting 10 minutes at 72°C. The amplified result was subjected to electrophoresis on 2% agarose gels and subsequently visualized using ethidium bromide. In each electrophoresis experiment, a set of standard molecular markers were included. Photographs of gels lighted by ultraviolet trans-illumination have been obtained.

RESULTS AND DISCUSSION

In the analysis of Inter Simple Sequence Repeat (ISSR), a total of three ISSR primers were utilized to screen and amplify bands. Subsequently, a selection of these amplified bands were chosen for further investigation. The size of the polymerase chain reaction (PCR) products varied between 100 base pairs (bp) and 10 kilo base pairs (Kbp). On average, there were 43.3 polymorphic bands seen for all the primers used.

Table (2) The quantity of amplified products

Primer Name	Total no. of band	No. of Polymorphic band	% polymorphism
ISSR13	7	13	53.84
UBC-878	2	6	33.33
UBC-880	4	11	36.36
Total	13	30	43.33

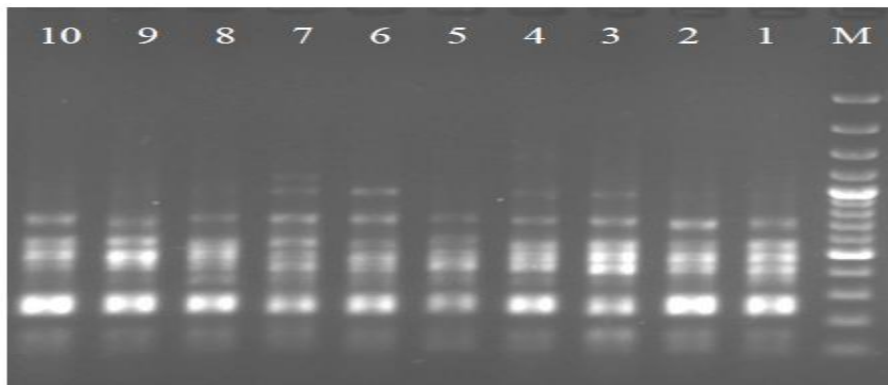


Fig. (1): The amplified result of ISSR13 was subjected to agarose gel electrophoresis.

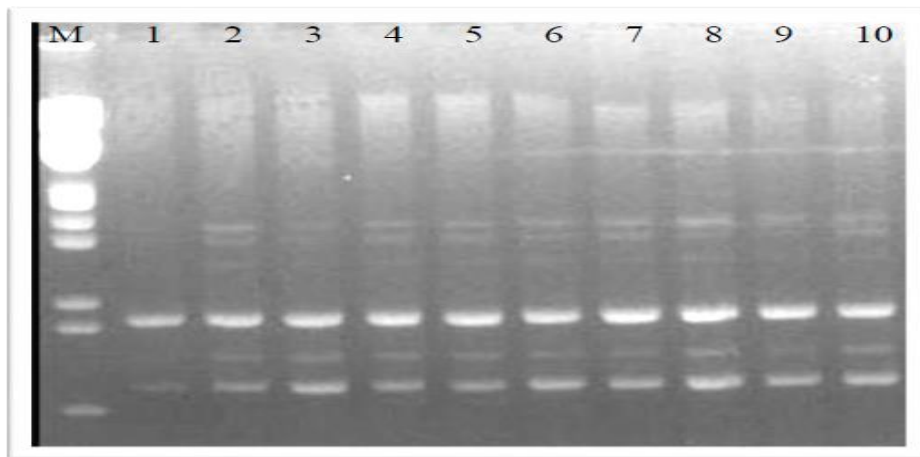


Figure 2 illustrates the agarose gel electrophoresis results of the amplified product obtained from the UBC-878 experiment.

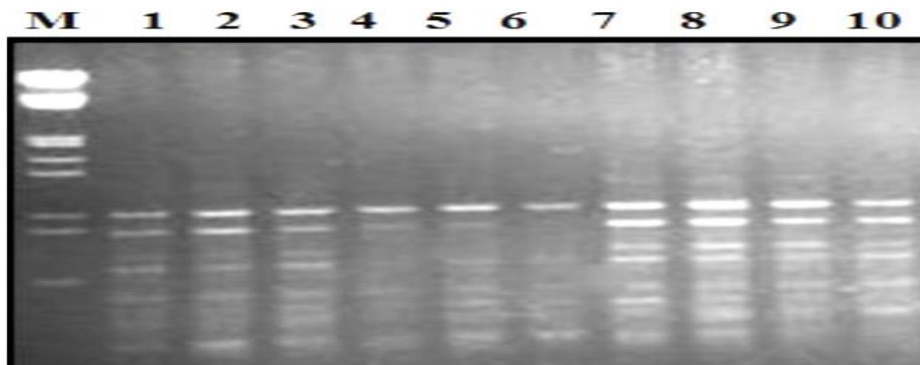


Figure 3 displays the agarose gel electrophoresis results of the amplified product obtained from UBC-880.



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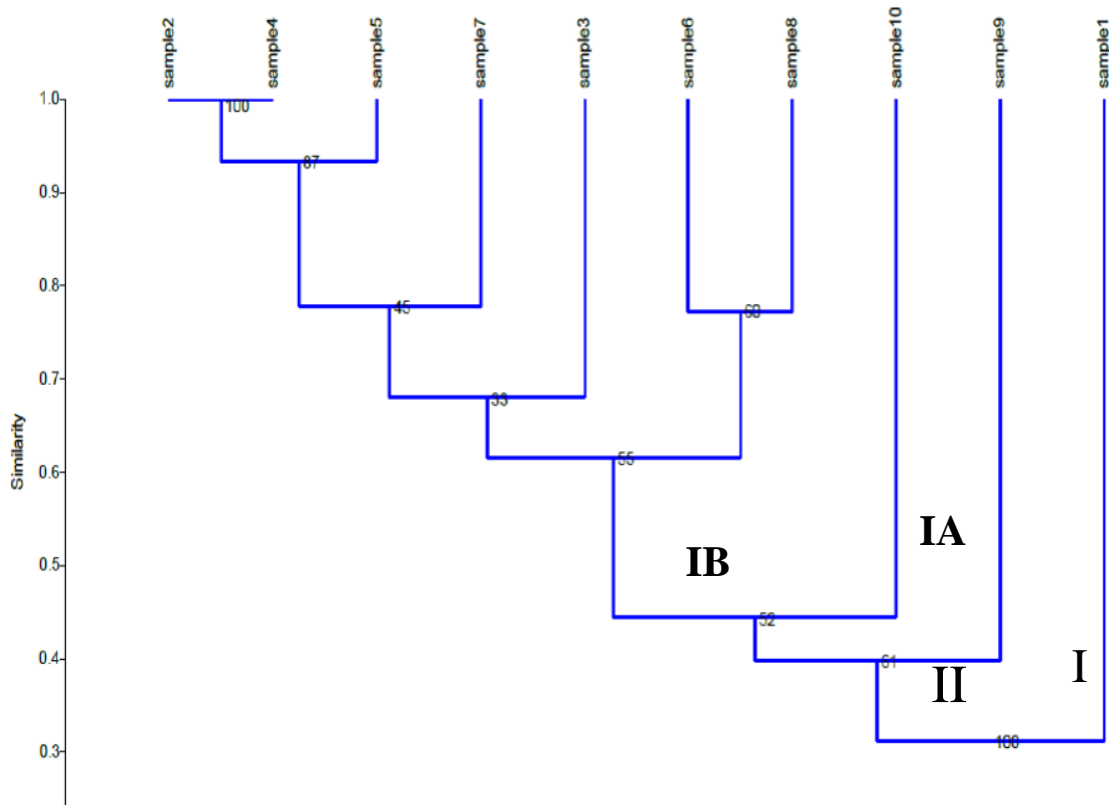


Figure (4) The present study used dendrogram analysis to illustrate the evolutionary diversity of ten specimens of *Vicia faba* L, utilizing ISSR markers for characterization purposes. Employing a triad of distinct primers



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Table 3 presents the genetic distance matrix, which is derived from ISSR markers using the Jaccard coefficient, for a total of 10 populations of Vicia faba.

sample1	sample2	sample3	sample4	sample5	sample6	sample7	sample8	sample9	sample10
sample1	0.000								
sample2	0.000	1.000							
sample3	0.000	0.000	0.000						
sample4	0.000	0.000	0.000	1.000					
sample5	0.000	0.000	0.000	0.241	0.241				
sample6	0.000	0.000	0.000	0.241	0.241	1.000			
sample7	0.000	0.000	0.000	0.000	0.000	0.000	0.000		
sample8	0.000	0.000	0.000	0.000	0.000	0.000	0.000	1.000	
sample9	0.000	0.000	0.000	0.000	0.000	0.024	0.024	0.550	0.550
sample10	0.000	0.000	0.000	0.000	0.000	0.024	0.024	0.550	0.550

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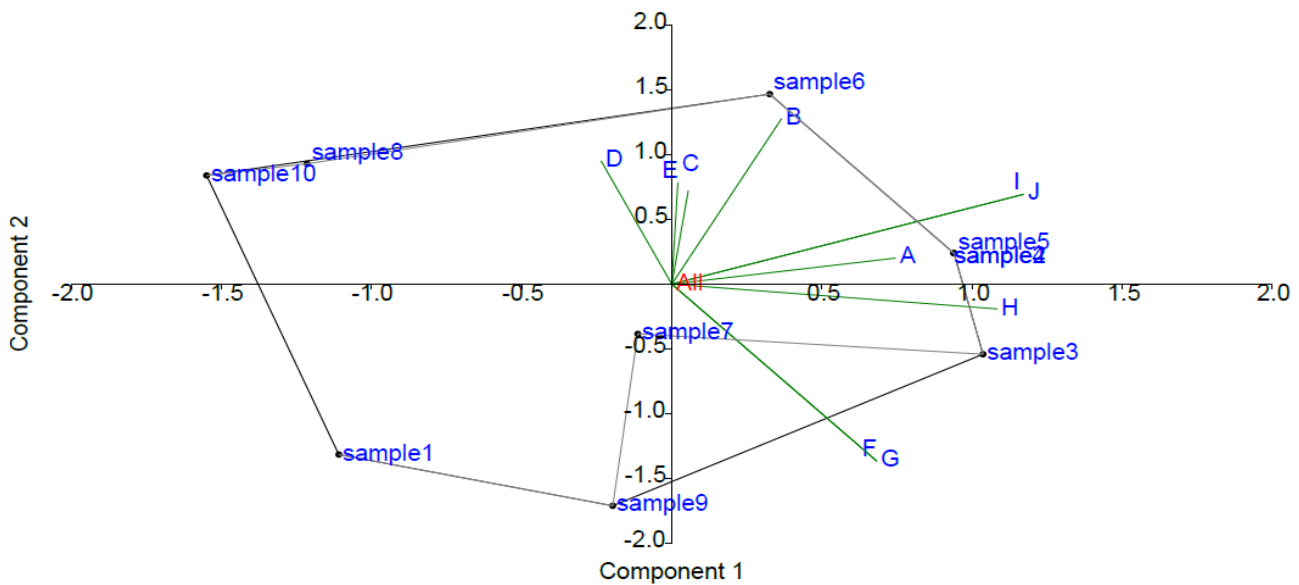


Fig.5 The present study employed three-dimensional principal component analysis (PCA) scaling to assess the genetic diversity of ten *Vicia faba L* genotypes, utilizing inter-simple sequence repeat (ISSR) markers.

The utilization of a set of three primers, which were meticulously constructed for ISSR analysis, facilitated the effective amplification of DNA through the polymerase chain reaction (PCR) technique. This amplification process yielded a collective count of 30 distinguishable DNA fragments that were visibly present in all genotypes. The overall proportion of polymorphic markers detected was 43.33%, and the mean proportion of polymorphism was also 43.33%. A dendrogram was created to represent the genotypes of *Vicia faba L* using cluster analysis. The present research was performed utilizing Jaccard's similarity coefficient matrices obtained from ISSR data. The UPGMA (Un-weighted Pair Group Method with Arithmetic Mean) algorithm was employed to generate a dendrogram using the similarity matrices provided in table 2[23].

The UPGMA (Un-weighted Pair Group Method with Arithmetic Mean) cluster analysis was employed to graphically depict the genetic distances among the ten fenugreek genotypes, as demonstrated in Figure 4. The dendrogram exhibited a bifurcation, dividing into two primary clusters. Cluster I was composed of a solitary genotype, while cluster II encompassed nine genotypes. Cluster II, which is the main cluster, was later divided into two sub-clusters known as IA and IB. It was observed that sub-cluster IA comprised a single genotype, while sub-cluster IB comprised eight genotypes.

The utilization of ISSR markers has proven to be successful in detecting genetic variation and establishing associations in many plant species [15,16]. Prior studies have demonstrated that these molecular markers exhibit exceptional utility in



evaluating genetic diversity, owing to their notable consistency and efficacy in detecting variations [14].

In the context of crop development initiatives, the acquisition of data pertaining to the extent and geographical spread of genetic variety, along with the interconnections among breeding materials, holds significant importance. The utilization of ISSR markers has proven to be effective in the identification of genetic diversity and relationships in different plant species [15]. Prior studies have demonstrated that these molecular markers possess notable utility in evaluating genetic variety owing to their strong consistency and robust ability to identify variation [17,18]. According to Bornet et al. [19] ISSR markers are characterized by their abundance, high reproducibility, high polymorphism, high informativeness, and rapidity of application. The ISSR system is considered to be one of the molecular marker systems that are specifically designed for focused analysis. It is known for its simplicity and reproducibility, as highlighted by Guo et al. [18].

The ISSR technique was devised as an approach similar to RAPD, although it has several advantages, notably the convenient assessment of several microsatellite regions dispersed throughout the genome. Consequently, this methodology is advantageous in the classification of genetic variation within or between species, as well as in the identification of agricultural plant varieties [20]. In actuality, the primers employed in this study enable the amplification of DNA sequences located between two closely positioned SSRs that are oriented in different directions. This amplification process yields a consistent pattern of genomic fragments. In addition, it should be noted that ISSR markers possess significant potential in the identification of polymorphism and the assessment of genetic diversity both at the inter- and intra-species levels.

The presence of polymorphism within a population can be attributed to the continued existence of genetic variants, as evidenced by the number of alleles at a certain genetic locus and their respective frequencies of distribution among individuals within the population. Heterozygosity refers to the probability of distinguishing between two alleles randomly selected from a population using the marker being examined. Therefore, it is suggested by Powell et al. [21] that an accurate quantitative assessment of the usefulness of the marker and the identified polymorphism can be determined by considering the average heterozygosity and the marker index.



CONCLUSION

In summary, the molecular markers have facilitated the estimation of the comprehensive genetic diversity in *Vicia faba L*, thereby unveiling genetic linkages based on scientific evidence. The present study also demonstrated that *Vicia faba L* in Iraq has a wide range of genetic diversity. Breeders have the ability to identify unique genotypes from various clusters and integrate them into their forthcoming breeding initiatives by utilizing the clustering pattern and genetic linkage established through ISSR markers.

Conflict of interests

There are non-conflicts of interest.

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الخلاصة

المقدمة تشكل البقوليات، المصنفة علمياً على أنها ثالث أكبر عائلة من النباتات الزهرية، ولا يتفوق عليها سوى البقوليات (Asteraceae Orchidaceae) ينتمي جنس *Vicia faba L.*، وهو جنس معتدل وعشبي بطبيعته، إلى قبيلة البقوليات Viciae من فصيلة Papilionoideae. وتعتبر البقوليات والحبوب هما أهم نباتين زهرين تستخدمان في الزراعة. فالباقوليات مفيدة كغذاء بشري وحيواني، وكمكونات لتحسين التربة في النظم الزراعية المختلفة.

المواد والطرق: تضمنت الدراسة الحالية استخراج الحمض النووي من أوراق نبات *Vicia faba L.*، يليه تضخيمه باستخدام ثلاثة بادئات لغرض إجراء تحليل ISSR بواسطة تفاعل البوليميراز المتسلسل

النتائج تم توليد ما يقارب 30 قطعة من الحمض النووي، والتي كان من الممكن بعد ذلك تقييمها عبر جميع الأنماط الجينية. تم تسجيل العدد الإجمالي للحزم متعددة الأشكال والنسبة المئوية المتوسطة لتعدد الأشكال بنسبة 33.43%.

الاستنتاج: تشير نتائج دراستنا إلى وجود درجة كبيرة من التنوع الوراثي ضمن نبات عزلات الباقلاء. يشير هذا النتيجة أيضاً إلى أن تقنية ISSR توفر معلومات أكثر شمولاً لتقييم التنوع الجيني نبات *Vicia faba L.*

الكلمات المفتاحية: نبات الباقلاء، التنوع الوراثي، ISSR، تفاعل البلمرة المتسلسل