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Genetic Studies on Breeding Faba Bean for Drought Tolerance 1- Genetic Variations

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ABSTRACT

Genetic relationships between six varieties and their fifteen hybrids of faba beans grown under the natural environmental condition at North Sinai Research Station (NSRS) were studied using ISSR molecular markers. Fifteen mer specific DNA primers were used. Out of used six arbitrary 15 mer primers, five were identified and detected polymorphism. The results indicated that there were some variations in banding patterns among these 6 parents which appeared a High level of polymorphism between it. The results of ISSR –PCR profiles of the studied faba bean genotypes for 15 F1 hybrids showed a High level of monomorphism was observed. The highest polymorphism with primer HB8 (GA) 6 GG at molecular weight 1100 bp. While the highest monomorphism with primers 17899B (CA) 6 GG at molecular weights (455, 749, 847 and1020 bp) bp, and HB9 (GT) 6 GG at molecular weights (500, 680, 1021 and 1299) bp. The genetic relationships based on ISSR markers were developed using SPSS computer program. Moreover, these molecular markers are useful tools for the breeder to decrese the time of breeding program. The results of genetic relationships showed that the genotypes were divided into two groups. Each group consists of the parents and their hybrids. The first group consist of the parents (Nubaria1 and Sakha2) and eleven hybrids of *Vicia faba* (L1, L2, L3, L4, L5, L6, L7, L8, L9, L10 and L11), while the second cluster included the rest of parents (Sakha1, Giza429, Giza716 and Giza2) and others hybrids (L12, L13, L14 and L15). Passed on the later the obtained results from ISSR analysis and the results of Dendrograms demonstrated the relationships refers to the cross direction in breeding program was done correctly. For this reason, results of positive and negative molecular size (pb) against different primers indicated that ISSR markers are effective in breeding programs for drought tolerance.

Key words: Faba bean, drought tolerance, molecular markers, dendrogram.

INTRODUCTION

Faba bean (*Vicia faba* L.) is the most important food legume in Egypt. The acreage and seed yields of faba bean vary according to seasons and locations. The national faba bean acreage over last three years (2001-2004) was 296,000 feddan with an average productivity of 8.5 ardabs/feddan. The yield instability is attributed to various biotic and environmental stresses (Darwish and Abdalla, 1997).

Faba bean is one of the most important pulse crops produced throughout the world, with roughly 46 million tons of production in 82% in the developing countries. The average seed grain yield of faba bean is around 1.8 t/ha. as compared with its potential (more than 3 t/ha) under farmers' conditions that employ improved crop management practices. Climatic variability (cold, heat and drought) and biotic factors (parasitic weeds and diseases) affect faba bean productivity. Drought is the most important limiting factor of faba bean in the Mediterranean region, mainly in low rainfall and marginal lands (Egypt, Syria, Tunisia, Morocco, Spain and Italy) (ICARDA, 2008).

Faba bean is a valuable protein-rich food that provides a large sector of the human populations in developing countries with a cheap protein source, thus partly compensating for the large deficiency in animal protein sources. In developed countries faba bean provides an alternative to soybean meal for animal feed, this being particularly important in the more industrialized countries. The world area devoted to faba bean is continuously in decline, falling from 3.7 m. ha. in 1979 / 81 to 2.4 m. ha. in 2000 / 01. This reduction is mainly attributed to the unreliable yields and the poor returns from the crop. It plays a significant role in improving the productivity of the soil in the cereal-based rotations where it serves as a break crop; yields of cereal crops following faba bean are improved and the needs for nitrogen fertilizer applications are reduced. In addition, an effective management of faba bean in crop rotations by reducing plant-available soil nitrogen before, during and after growing faba bean could achieve maximum symbiotic activity, low levels of nitrogen leaching and high yield of the succeeding non-legume crops.

The Methods of plant breeding and genetic modification can speed the transition to more efficient water use and considerable success has already been achieved. Hybrid seed likely to be expensive and the economics of commercial production of hybrid faba bean have not yet been worked out. The economic feasibility would be considerably improved if sufficient heterosis were retained in the F2 generation to make its production of value particularly under the stress conditions. After progress of molecular marker techniques, new reliable tools that are neither affected by the surrounding environment, nor by growth stage of

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the plant (as in case of morphological characters) became available for the breeder. These can be applied for organizing germplasm, identification of cultivars, assisting in the selection of parents for hybridization and reducing the number of accessions needed to ensure sampling a broad range of genetic variability.

MATERIALS AND METHODS

Materials

A half diallel cross set involving the six parents were made in 2006/07 season under open field conditions, under normal conditions of North Sinai Research Station (NSRS), Desert Research Center (DRC).

In 2007/08 season, two experiments were conducted; each experiment included the six parents and their 15 F1 hybrids, which were sown on November 1st, 2007 in a randomized complete block design (RCBD) with three replications. The 1st experiment was normally irrigated at 60-70% of field capacity (FC) in addition to the rainfall, the 2nd experiment, at 40-50% FC with the rainfall. All other agricultural practices were carried out as usual in the conventional faba bean fields. Then, ISSR analysis was preformed on twenty one DNA samples (parents and hybrids) representing faba bean genotypes.

A. Parents

Six varieties of faba bean (Nubaria1 (P1), Sakha2 (P2), Sakha1 (P3), Giza429 (P4), Giza716 (P5) and Giza2 (P6).) obtained from Field Crops Research Institute (FCRI), Agriculture Research Center (ARC), Giza, Egypt

B. Hybrids

The Pedigree of fifteen hybrids genotypes were (Nubaria1 x Sakha2, Nubaria1 x Sakha1, Nubaria1 x Giza429, Nubaria1 x Giza716, Nubaria1 x Giza2, Sakha2 x Sakha1, Sakha2 x Giza429, Sakha2 x Giza716, Sakha2 x Giza2, Sakha1 x Giza429, Sakha1 x Giza716, Sakha1 x Giza2, Giza429 x Giza716, Giza429 x Giza2 and Giza716 x Giza2).

Methods

A. DNA extraction

From the field experimental site, young leaves of each genotype were randomly collected from ten plants and then sample of one gram was treated with liquid nitrogen and transferred to Biotechnology Lab., NSRS, DRC, Egypt for DNA extraction according to the method of Junhans and Metzlatt (1990).

B. DNA amplification

Protocol for PCR-ISSR reaction was conducted using 18-20-base oligonucleotide primers (Operon technologies Inc., U.S.A) according to Nagaoka and Ogihara (1997) as shown in Table (1).

Table (1): Nucleotide sequences of five specific primers used in this study.

No.	Primer code	Primer sequence
1	17899A	(CA)6 AG
2	17899B	(CA)6 GG
3	HB4	(GACA)4
4	HB8	(GA)6 GG
5	HB9	(GT)6 GG

C. Gel electrophoresis

A volume of 20 µl of PCR- products were resolved in 1.5% ultra pure (GIBCOBRL) agarose gel electrophoresis with 1x TAE running buffer. The run was performed at 80 V for 100 min and the gel was stained with ethidium bromide. A marker of 1 Kb plus DNA Ladder 1µg /µl that contains a total of twenty bands ranging from100 to 10000 bp was used. Bands were visualized on UV – transilluminator and photographed by Polaroid camera. Gels were analyzed by gel documentation system. Amplified products were visually examined and the presence or absence of each size class was scored 1 or 0, respectively.

RESULTS AND DISCUSSION

1. ISSR-PCR amplification analysis

Inter-Simple Sequence Repeats (ISSRs) has only recently been developed as an anonymous, RAPDs-like approach that accesses variation in the numerous microsatellite regions dispersed throughout the various genomes (particularly the nuclear genome) and circumvents the challenge of characterizing genotype loci that other molecular approaches require (Junhans and Metzlat, 1990). Microsatellites are very short (usually 10-20 base-pair) stretches of DNA that are "hypervariable", expressed as different variants within populations and among different species. Studies of the heritability of ISSR locus have demonstrated an exceedingly close approximation to classic Mendelian ratios. Extremely high variability and high “mapping density” as compared with RFLP and RAPD data make these new dominant, microsatellite-based molecular markers ideal for producing genetic maps of genotype (Nagaoka and Ogihara 1997). ISSR analysis was preformed on twenty one DNA samples representing faba bean genotypes using five primers composed of short tandem repeat sequences with or without anchor.

(A) Primer 17899A

The results of primer 17899A are illustrated in figure (1A) and Table (2); it gave eight polymorphic and two monomorphic bands with different fragment sizes which range from 425 to 1757 bp. Two common bands were observed in all genotypes at 934 bp and 1280 bp. Sakha1 could be distinguished from all other genotypes by the presence of one band at fragment size of 1757 bp.

Finally, a band with a fragment size of 1314 bp was present in Giza429 and their hybrids (L3, L7, L10, L13 and L14) and disappeared in all genotypes. This indicated that the used parents are genetically varied.

(B) Primer 17899

The results of primer 17899B are demonstrated in Figure (1B) and Table (3). It gave five polymorphic and three monomorphic bands with different fragment sizes which ranging from 455 to 1860 bp. Three common bands were observed in all genotypes at 455, 749 and 1020 bp. This primer showed that the parents and their 15 hybrids have a good degree of similarity. This similarity refers to that the hybridization process was done correctly in breeding program.

(C) Primer HB4

The results of primer HB4 are conducted in Figure (1C) and Table (4).It gave five polymorphic and tow monomorphic bands with different fragment sizes which ranging from 477 to 1286 bp. Two common bands were observed in all genotypes at 477 bp and 738 bp. Finally, a band with a fragment size of 12864 bp was present in Sakha1 and their hybrids (L2, L6, L10, L11 and L12) and disappeared in the rest genotypes. Commons of all the parents with their hybrids in many of bands is an indicator that the direction of hybridization during the breeding program was done correctly. These results are agreed with Lanza *et al.* (1997) on all genotypes of Faba bean.

(D) Primer HB8

The results of primer HB8 are demonstrated in Figure (1D) and Table (5). It gave six polymorphic and one monomorphic band with different fragment sizes which ranging from 529 to 1537 bp. One common band was observed in all genotypes at 1100 bp. This indicates that the used parents which are genetically varied so, they are good parents for produced many of genotypes, may be more tolerant for drought.

(E) Primer HB9

The results of primer HB9 are illustrated in Figure (1E) and Table (6). It gave one polymorphic and four monomorphic bands with different fragment sizes which ranging from 500 to 1299 bp. Four common bands were observed in all genotypes at 500 bp, 680, 1021 and 1299bp. This primer had the most similarity between the used genotypes relative to the other used primers in current study.

2. Molecular levels of polymorphism

Five preselected ISSR primers were used in the present study to identify the twenty one *Vicia faba* genotypes as shown in Table (7). Twenty one monomrphic and Twenty five polymorphic distinct fragments (67.5% of polymorphism) were revealed in the 21 tested genotypes with these primers. The results showed that primers 17899A, HB4 and HB8 were highly polymorphic (from 71.4% to 80% of polymorphism). Moreover, primer 17899B was medial between polymorphic and monomorphic (50% of monomorphism to 50% of polymorphism). On the other hand, primer HB9 was highly monomorphic with about 20% polymorphism.

In general, the results indicated that ISSR markers gave adequate distinctions among Vicia faba genotypes. These results were in partial agreements with the finding of Wolfe and Liston (1998) who reported that ISSR markers have recently become widely used in population studies because due to their high variability, require less investment in time, money and labor than other methods. Wang and Fan Ming (1998) successfully used five ISSR primers to evaluate 90 accessions of pepper germplasm collected from 16 countries of Europe. Pharmawati *et al.* (2005) evaluated the genetic variations among 30 *Leucadendron* cultivars using 64 ISSR primers. They reported that ISSR profiling is a powerful method for the identification of *Leucadendron* cultivars. Finally, Abdel-Tawab *et al*. (2007) used ten ISSR primers to differentiate among four *Mentha* and three *Ocimum* sp and reported detection of high polymorphism between them.

3. Genetic relationships among *Vicia faba* genotypes

Based on ISSR marker polymorphisms, similarity matrix was developed by SPSS computer package (Table 8). The analysis was based on the number of markers that were different between any given pair of genotypes. The closet relationship was scored between

Table (2): DNA polymorphism in twenty one faba bean genotypes using PCR with primer (17899A).

Genotypes bp	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
1757	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1314	0	0	0	1	0	0	0	0	1	0	0	0	1	0	0	1	0	0	1	1	0
1280	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
1153	1	1	0	0	1	0	1	1	1	1	1	1	1	1	1	1	1	0	0	0	1
934	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
787	1	1	0	0	0	0	1	1	1	1	1	1	1	1	1	1	0	0	0	0	1
673	1	1	1	1	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1
595	1	1	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0
528	0	1	1	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
425	0	0	1	1	0	0	1	1	0	1	1	1	1	1	1	1	1	0	0	0	1

Table (3): DNA polymorphism in twenty one faba bean genotypes using PCR with primer (17899B).

Genotypes bp	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
1860	0	0	1	0	0	0	1	1	1	1	1	0	1	1	1	1	0	1	1	0	0
1462	1	0	0	0	0	0	1	1	1	1	1	0	1	1	1	1	1	0	0	0	0
1020	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
847	1	1	1	1	1	1	0	1	0	1	0	0	1	0	0	0	0	0	0	0	0
749	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
626	1	0	1	1	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
550	0	1	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
455	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1

Table (4): DNA polymorphism in twenty one faba bean genotypes using PCR with primer (HB4).

Genotypes bp	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
1286	0	0	1	0	0	0	0	1	0	0	0	1	0	0	0	1	1	1	0	0	0
1031	0	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0
936	1	1	1	0	0	0	0	1	1	1	1	1	1	1	1	1	0	1	0	1	0
738	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
672	0	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	0	1	0
555	0	1	0	1	0	0	0	1	1	1	1	0	0	1	0	0	0	0	0	1	0
477	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1

Table (5): DNA polymorphism in twenty one faba bean genotypes using PCR with primer (HB8).

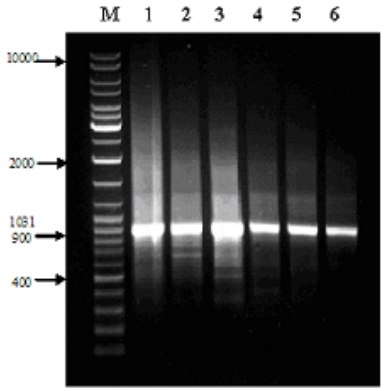
Genotypes bp	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
1537	0	0	1	1	1	1	1	1	1	0	1	0	1	1	1	1	1	0	1	1	1
1200	0	0	0	0	0	1	0	0	1	1	0	0	1	0	1	0	0	0	0	0	0
1100	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
1048	1	1	1	0	1	1	0	0	1	1	1	0	1	1	1	1	1	0	1	1	1
861	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	0	1
758	1	0	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
529	0	1	1	1	1	1	1	1	1	1	1	0	1	1	1	1	1	0	1	1	1

Table (6): DNA polymorphism in twenty one faba bean genotypes using PCR with primer (HB9).

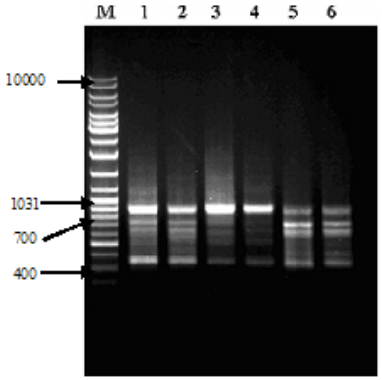
Genotypes bp	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
1299	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
1129	0	0	0	1	0	0	0	1	0	1	0	0	1	1	0	1	0	0	0	0	0
1021	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
680	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
500	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1

Table (7): Levels of polymorphism detected with each ISSR primer in *Vicia faba* genotypes.

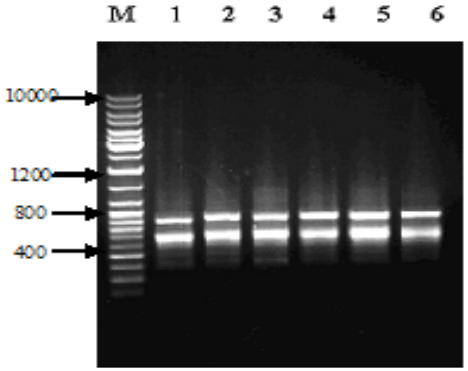
Primer Number	Primer code No.	Monomorphic bands	Polymorphic bands	Total	Polymorphism / primer %
1	17899A	2	8	10	80%
2	17899B	3	5	8	62.5%
3	HB4	2	5	7	71.4%
4	HB8	1	6	7	85.7%
5	HB9	4	1	5	%20
Total band numbers detected for all primers		12	25	37	%67.5



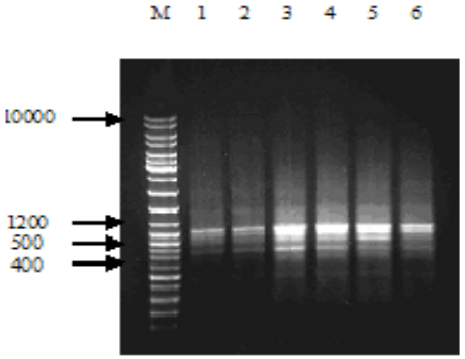
A: (17899A)



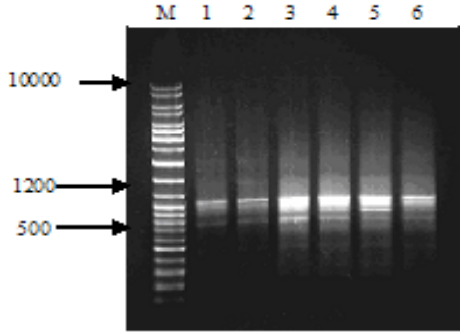
B: (17899B)



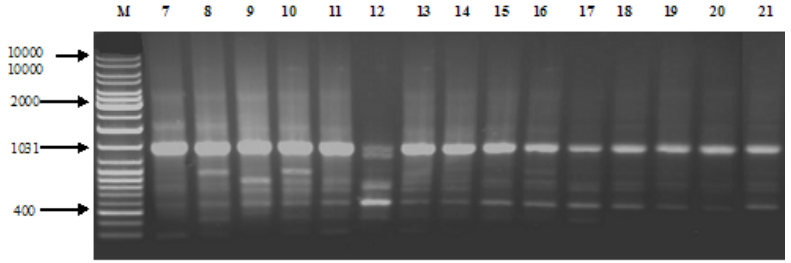
C: (HB4)



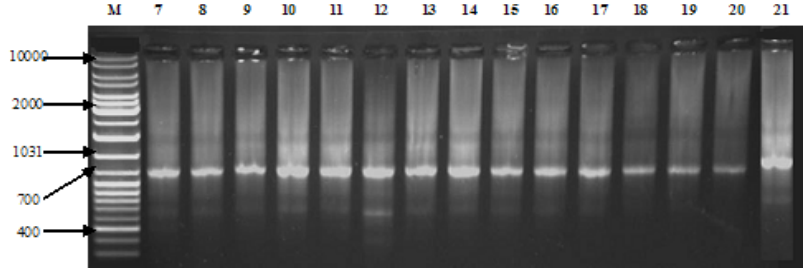
D: (HB8)



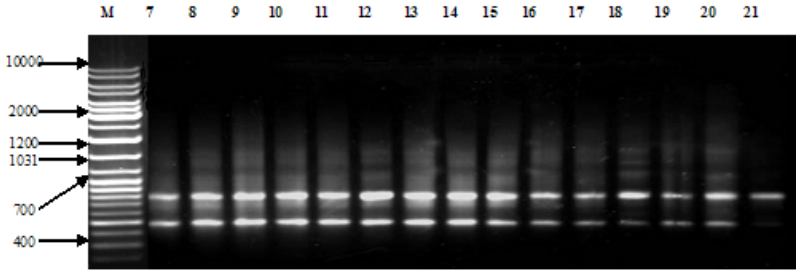
E: (HB9)



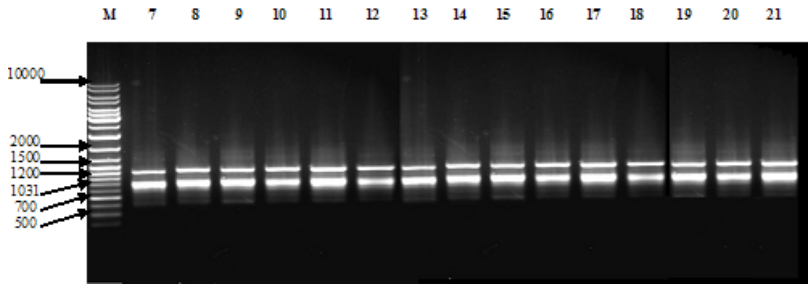
A: (17899A)



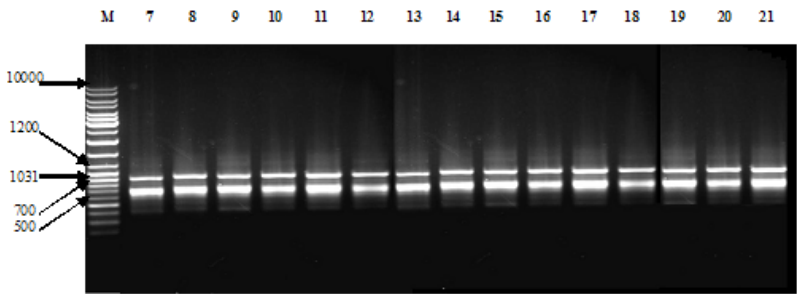
B: (17899B)



C: (HB4)



D: (HB8)



E: (HB9)

Figure (1): DNA polymorphism of twenty one faba bean genotypes : (1, (P1); 2, (P2); 3, (P3); 4, (P4); 5, (P5); 6, (P6); 7, (P1 x P2); 8, (P1 x P3); 9, (P1 x P4); 10, (P1 x P5); 11, (P1 x P6); 12, (P2 x P3); 13, (P2 x P4); 14, (P2 x P5); 15, (P2 x P6); 16, (P3 x P4); 17, (P3 x P5) ; 18, (P3 x P6); 19, (P4 x P5); 20, (P4 x P6) & 21, (P5 x P6).) using5 specific primers (A,17899B; B,17899B; C, HB4; D,HB8 & E,HB9).

Table (8): Similarity matrices among the twenty one *Vicia faba genotypes* based on ISSR analysis.

	Nubaria 1	**Sakha 2**	**Sakha 1**	**Giza 429**	**Giza 716**	**Giza 2**	**L1**	**L2**	**L3**	**L4**	**L5**	**L6**	**L7**	**L8**	**L9**	**L10**	**L11**	**L12**	**L13**	**L14**
Sakha2	0.568																			
Sakha1	0.297	0.297																		
Giza 429	0.189	0.189	0.459																	
Giza 716	0.405	0.405	0.459	0.568																
Giza 2	0.243	0.351	0.405	0.514	0.84															
L1	0.189	0.189	0.243	0.351	0.24	0.189														
L2	0.243	0.351	0.297	0.514	0.19	0.135	0.73													
L3	0.297	0.405	0.351	0.243	0.24	0.297	0.57	0.622												
L4	0.351	0.459	0.297	0.405	019	0.243	0.51	0.784	0.73											
L5	0.351	0.459	0.351	0.405	0.3	0.243	0.73	0.784	0.838	0.784										
L6	0.459	0.459	0.297	0.405	0.3	0.243	0.62	0.568	0.514	0.568	0.676									
L7	0.297	0.297	0.459	0.351	0.24	0.297	0.57	0.73	0.784	0.838	0.73	0.514								
L8	0.297	0.405	0.351	0.459	0.24	0.189	0.68	0.838	0.784	0.838	0.946	0.622	0.784							
L9	0.297	0.297	0.459	0.243	0.24	0.297	0.68	0.622	0.892	0.73	0.838	0.622	0.892	0.784						
L10	0.297	0.297	0.459	0.351	0.24	0.189	0.68	0.73	0.784	0.73	0.838	0.622	0.892	0.892	0.832					
L11	0.459	0.459	0.405	0.405	0.41	0.351	0.62	0.459	0.514	0.459	0.676	0.568	0.514	0.622	0.622	0.622				
L12	0.297	0.297	0.243	0.135	0.14	0.189	0.46	0.297	0.243	0.189	0.297	0.514	0.135	0.243	0.243	0.243	0.297			
L13	0.189	0.297	0.351	0.351	0.46	0.514	0.46	0.189	0.243	0.081	0.297	0.189	0.135	0.243	0.243	0.243	0.514	0.57		
L14	0.189	0.297	0.243	0.351	0.46	0.514	0.24	0.189	0.243	0.081	0.297	0.189	0.027	0.243	0.135	0.135	0.297	0.57	0.68	
L15	0.351	0.459	0.297	0.405	0.51	0.459	0.51	0.243	0.297	0.243	0.459	0.459	0.297	0.405	0.405	0.405	0.676	0.3	0.73	0.514

the two different genotypes of faba bean (L5 and L8) of hybrids (similarity of 0.946), followed by L3 and L9 genotypes and between L9 and L10 genotypes of hybrids (similarity of 0.892). On the other hand, the most distant relationship was scored between L7 and L14 genotypes of hybrids (Similarity of 0.03). The Dendrogram (Fig. 2) classified the twenty one genotypes into tow main clusters. The first cluster comprised of the first parents (Nubaria1 and Sakha2) and the eleven hybrids of *Vicia faba* (L1, L2, L3, L4, L5, L6, L7, L8, L9, L10 and L11), while the second cluster included the rest of parents (Sakha1, Giza429, Giza716 and Giza2) and others hybrids (L12, L13, L14 and L15). The first cluster was separated into two sub-clusters. The first sub-cluster included only the parents (Nubaria1 and Sakha2) of *Vicia faba* and their hybrids (from L1 to L11). On the other hand, the second cluster comprised the rest of parents (Sakha1, Giza429, Giza716 and Giza2) and their hybrids (from L12 to L15).

In general, the relationships among *Vicia faba* genotypes derived from ISSR analysis were in partial agreement with the knownlineage of these genotypes. In a comparable study, Gilbert *et al.* (1999) used ISSR markers to reveal the genetic relationships within and between accessions held in a collection of lupin germplasm. Zavodna *et al*. (2000) successfully used inter- simple sequence repeats (ISSR) to distinguish among commercial lentil cultivars (Lens cultivars). Moreover, Alexander (2002) successfully used ISSR marker to determine the levels and distribution of genetic relationships within and among populations of *Astragalus oniciformis*. Hassan (2005) reported that ISSR markers were a good choice for the evaluation of diversity and assessing the genetic relationships between moringa and mentha genotypes with a high accuracy. In addition, Abdel-Tawab *et al.* (2007) used 10 ISSR primers to differentiate among four *Mentha* and three *Ocimum* sp and reported that dendrogram based on ISSR markers successfully separated the seven species into two main clusters of the two *Mentha* and *Ocimum genera*.

The dendrogram (Fig. 2) classified the twenty one genotypes into two main clusters. Moreover, the first cluster was separated into two sub-clusters. Within the first sub-cluster of the first cluster, all parents were grouped together in separately sub-clusters. The genotypes (L1, L2, L3, L4, L5, L6, L7, L8, L9, L10 and L11) which were resulted from the parents (Nubaria1 and Sakha2) in main cluster. On the other hand, all other parents (Sakha1, Giza429, Giza716 and Giza2) with their hybrids (L12, L13, L14 and L15) separately in other cluster. These results are agreed with those of Lara *et al*. (2003) studied the genetic diversity among four populations of *Psychotria acuminate* as medicinal plant. They showed that high genetic diversity were detected among populations based on both RAPD and ISSR methods. Zhou *et al*. (2004) who reported that RAPD and ISSR markers are suitable for assessment of genetic diversity of *Rehmannia glutinosa* sp germplasm. Said (2005) reported that RAPD and ISSR markers are useful tools to asses the genetic variations in caper and arghel species for the conservation of its germplasm. El Ramah (2006) suggested that RAPD and ISSR markers are the best choice for the evaluation of diversity and assessing the genetic relationships between *P. tortuosus* and Fennel genotypes with high accuracy. Abdel-Tawab *et al.* (2007) reported that phylogenetic relationships based on RAPD and ISSR markers succeeded in separating two species of *Mentha* and *Ocimum* genera into two main clusters.

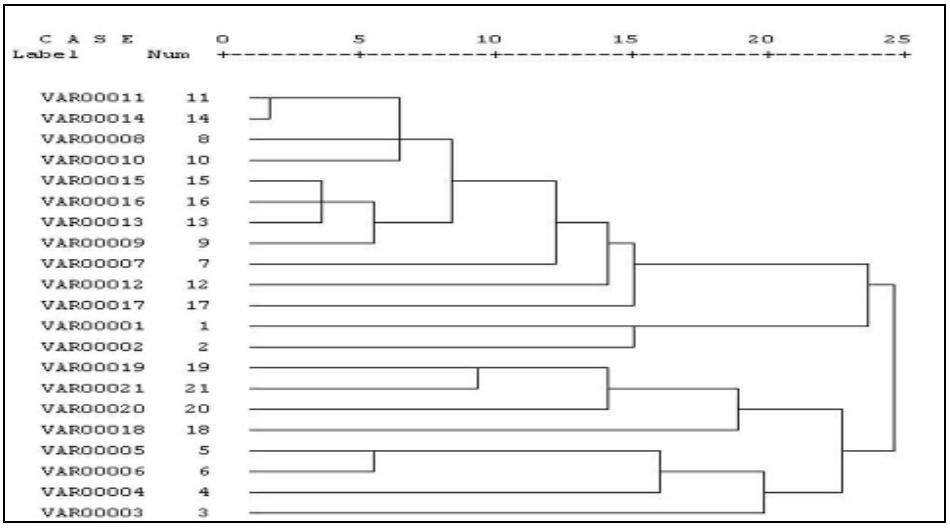


Figure (2): Dendrogram showing the classification of the twenty one genotypes into two main clusters.

Conclusion

From the obtained results the conclusions could be summarized as follows: (1) The results from ISSR analysis refers to the cross direction in breeding program was done correctly, High heritability values were found high rates of success in recovering the desired genes in future generations. Also, this high estimates indicate that the selection for this character in early segregated generation could be possible. While delaying it would be more available, (2) Plant breeders should have the ability to use molecular marker tools for selection in breeding programs for drought tolerance.

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دراسات وراثية على تربية الفول البلدى لتحمل الجفاف
1- الاختلافات الوراثية الجزيئية

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الملخص العربـــى

تم إستخدام ال ISSR لدراسة الاختلافات الوراثية على مستوى ال DNA بين ستة أصناف محلية من الفول البلدى وبعضها البعض، وبين خمسة عشر هجين فردى مستنبطة من تلك الأباء بنظام التهجين النصف دائرى. وقد إتفقت النتائج التى تم الحصول عليها والخاصة بالعلاقة الوراثية بين الهجن الفردية الناتجة فى الجيل الاول مع الاباء المستخدمة فى التهجين، مما يدل على صحة إتجاة التربية. اظهرت نتائج دراسة التراكيب الوراثية للاباء وجود تباعد وراثى بين تلك الاباء، والذى يمكن الاعتماد عليه فى التربية لتحسين الفول البلدى. كما تم دراسة علاقة القرابة الوراثية بين الخمسة عشر تركيباً وراثياً من الفول البلدى (الهجن الفردية)، ووجدت درجات قرابة وراثية عالية بين الهجن الناتجة من أباء مشتركة ، فى حين إنخفضت درجة القرابة بين الهجن الناتجة عن أباء مختلفة. ويمكن الإستفادة من هذه التقنية ISSR فى الدراسات المستقبلية فى الإنتخاب لصفة تحمل الجفاف وغيرها من الصفات فى الجيل الثانى وما يليه.