Journal of Agricultural Chemistry and Biotechnology

Molecular Assessment of Ten Faba Bean Genotypes Dissimilar in Bean Yellow Mosaic Virus Susceptibility

Salim, T. M. S.*

Genetic and Genetic Engineering Department, Faculty of Agriculture, Benha University, Moshtohor 13736, Qaliuobia, Egypt

Cross Mark

ABSTRACT

The point of this investigation was to decide the hereditary inconstancy and break down the connection between four faba bean cultivars and six promising lines utilizing inter simple sequence repeats (ISSR) markers. Faba bean genotypes were gathered by their resistance to the bean yellow mosaic virus infection. With the acquired band registration, a paired data matrix was built to perform the comparing statistical analysis. The used ISSR markers created 148 fixed and repeatable bands, of which 75 were polymorphic. The values of resolution power (Rp), polymorphic information content (PIC), and marker index (MI), uncovered that primer ISSR-14 was the most effective to break down hereditary changeability with estimations of 5.8, 0.80, and 6.40, respectively, followed by primers ISSR-6 and ISSR-18. Genetic distances swayed somewhere in the range of 0.78 and 0.91, and checked the groupings saw in the dendrogram, which demonstrates high change at the degree of DNA among the investigated genotypes, watching six characterized gatherings as indicated by UPGMA examination. In the examination of fundamental segments, the enrolled groupings were dictated by the source of the origin of the gathering. The utilization of ISSR markers was effective to describe at the degree of DNA the evaluated bean genotypes, showing the presence of inconstancy, the recognized differentiating promotions can be used in hereditary improvement projects planned for settling the requirements of the producers.

Keywords: Vicia faba, Bean yellow mosaic virus, susceptibility, ISSR.

INTRODUCTION

Faba bean is commonly utilized as human sustenance in creating nations and as animal nourishment, in both industrialized and developing countries. It remains the most significant leguminous sustenance crop in Egypt. It very well may be utilized likewise as vegetables, green or dried, new or canned. The faba bean can be considered as a supplement to meat or even as an alternative. Numerous investigators recorded that diseases found on faba bean was considered the most damaging and cause significant misfortunes in yield. Among these diseases, viral ailments can influence faba bean plants which cause critical monetary misfortunes and yield decrease. Certain infections cause's critical monetary misfortunes and yield decrease, which could be up to 30% on susceptible faba bean genotypes as pointed out by (Khalil and Erskine 2001). Among faba bean infections, bean yellow mosaic virus (BYMV) consider as one of the most decimating infections influencing faba bean plants in Egypt. BYMV (*Family Potyviridae*, sort Potyvirus) is an aphid transmitted infection in non-constant mode and has a wide range of hosts (Jones 2004 and 2005). Foundational side effects brought about by BYMV disease don't execute faba bean plants, however, can possibly spread quicker and further into the plant, causing more prominent yield decrease, notwithstanding instigating milder manifestations. Due to the agronomic and economic interest this species represents, the objective of this work.

The aim of this search was to determine the genetic diversity and relevance between 10 faba bean genotypes via methods of ISSR molecular markers, in perspectives on recognizing and choosing genetically differentiating accessions. The results of this work will allow using the detected genetic variability and incorporate it in phenotyping and genotyping, schemas aimed at increasing production, producing resistance or tolerance to abiotic pressure, and in any event, making high-performing and stable materials reasonable for different abiotic stresses.

MATERIALS AND METHODS

In this study, seeds from six faba bean populations were collected because of their best agro-morphological characteristics, and four varieties generated by the Institute of Agricultural Research Center, Cairo, Egypt. Pedigree and resistance degree to BYAV of the 10 assessed accessions were summarized in Table 1.

Table 1. Pedigree and resistance degree of ten faba bean genotypes utilized inside this investigation.

No.	Genotypes	Pedigree	Artificial infection
1	Giza 843	(561/2076/85 SKH X 461/485/83)	Mottle
2	Sakha 1	Giza 716 X 620/283/85	Mottle – Vein Yellowing
3	Sakha 4	Sakha 1 X Improved Giza 3	Mosaic - Deformation
4	Giza 716	Crossing (416/842/83 X 503/453/83)	Mottle – Vein Yellowing
5	X-2338	Misr 1 X Misr 3	Mottle
6	X-2339	(X-1714) X Misr 3	Mottle – Vein Yellowing
7	X-2340	(X-1714) X Giza 40	Mosaic–Vein Yellowing-Deformation
8	X-2341	Misr 1 X Nubaria 1	Mottle – Vein Yellowing
9	X-2342	Misr 3 X Giza 40	Mottle – Vein Yellowing
10	X-2343	Nubaria 1 X Giza 40	Mosaic

Reference: El-Sayed *et al.,*2019

ISSR-PCR Reaction:

A set of 12 primers ISSR (Table 2) was used in the detection of polymorphism. The amplification reaction was achieved in 25 µl reaction volume include 1X PCR buffer, 1.5 mM MgCl2, 0.2 mM dNTPs, 1 µM primer, 1 U Taq DNA polymerase and 30 ng template DNA.

Five grams of fresh foliar tissue from seedling of two weeks ago, healthy and without any mechanical damage were randomly selected and weighed for each of the 10 populations.

DNA isolation was accomplished according to the sodium dodecyl sulfate strategy by Dellaporta *et al.* (1983) with minor modulations, such as supplement of 0.5 mM ascorbic acid, as suggested by Hoelzel (1992) to minimize oxidation. The goodness of DNA was estimated by gel electrophoresis at 0.8% agarose. A set of 10 ISSR primers were utilized in this examination.

Table 2. Names and sequences of 12 ISSR primers employed within this study.

S. n.	Name Primer	Sequence 5'-3'
1	ISSR- 2	5'-AGAGAGAGAGAGAGAGAGYG-3'
2	ISSR- 5	5'-GTGTGTGTGTGTGTGTGTYG-3'
3	ISSR- 6	5'-CGCGATAGATAGATAGATA-3'
4	ISSR-7	5'-GACGATAGATAGATAGATA-3'
5	ISSR- 10	5'-GACAGACAGACAGACAAT-3'
6	ISSR- 11	5'-ACACACACACACACACYA-3'
7	ISSR- 12	5'-ACACACACACACACACYC-3'
8	ISSR- 13	5'-AGAGAGAGAGAGAGAGYT-3'
9	ISSR- 14	5'-CTCCTCCTCCTCCTCTT-3'
10	ISSR- 15	5'-CTCTCTCTCTCTCTCTRG-3'
11	ISSR- 16	5'-TCTCTCTCTCTCTCTCA-3'
12	ISSR- 18	5'-HVHCACACACACACACAT-3'

A: Adenine, T: Thymine, G: Guanine and C: Cytosine, Y: (C or T), V: (A or C or G), H: (A or C or T)

DNA Intensification were carried out in a thermocycler (TC-412, Bibby Scientific Limited, Stone, Staffordshire, UK) with a cycle for pre-denaturalization at 93 ℃ for 20 s, followed by 40 rounds as the denaturalization step at 94 ℃ for 20 s; one adjusting stage at temperatures had been defined according to the primer to amplify for 1 min, and a polymerization step at 72 ℃ for 20 s; at long last an extra cycle to wind up polymerization at 72 ℃ for 6 min. Product of the amplification of this predestined primers were separated in a horizontal electrophoresis chamber contain ultrathin agarose gel at 2%. At a later time, they were soaked in a solution of 0.5 μg mL^{-1} of ethidium bromide, and at long last visualized and analyzed in a high-performance UV transilluminator (Analytik Jena AG, Jena, Germany). The envisioned bands were enlisted and identified, taking into account the 1 presence and 0 nonappearances.

Just proportionate and reproducible bands were specified to run the compatible statistical analyses. DNA polymorphic bands were registered as discreet variables considering "1" presence and "0" absence to construct a binary data matrix. Finally, data were handled in Free Tree statistics software Version 0.9.1.5 (Pavlicek *et al.*, 1999) to manufacture a genetic distance matrix using Dice coefficient, otherwise called similarity coefficient Nei and Li (1979). Generated matrix was analyzed according to the Unweighted Pair Group Method with Arithmetic Mean (UPGMA), to texture a dendrogram with 1000 bootstrap replicates. Tree View software was used to visualize the obtained dendrogram.

The informational confidence of primers to distinction between diverse genotypes was examined by means of the estimation of their Polymorphic Information Content (PIC), Marker Index (MI) and Resolution power (Rp). PIC was calculated using the formula depicted by Roldán-Ruíz *et al.* (2000), which is: $PIC_i = 2f_i (1 - f_i)$, where PIC_i is the polymorphic information content of the first i, f_i is the frequency of the present bands, and $(1 - f_i)$ represents the frequency of the absent bands. Considering that the highest value of PIC for dominant markers is 0.5 according to De Riek *et al.* (2001). MI was calculated following Prevost and Wilkinson (1999), as $MI = PIC \times$ number of polymorphic bands, and Rp according to the formula by Gilbert *et al.* (1999),

$Rp = \Sigma$ Ib, where lb represents the information of the band, which was calculated with: $Ib = 1 - (2 \times | 0.5 - p |)$, where p is the proportion of accessions that contain bands and l represents the percentage of polymorphic bands (PPB). The origin of the genotypes was also tacked into account to check the possibile effect of genetic grouping. The Principal Component Analysis (PCA) was gained by analyzing the correlation of the binary data matrix in GenAlEx 6.501 software (Peakall and Smouse, 2006; 2012) and explicated according to the three main first coordinates.

RESULTS AND DISCUSSION

Polymorphism of ISSR markers

From numerous examined primers just 12 amplified worthily repeatable and clear polymorphic bands (Figure 2). Nucleotide sequences, moreover names of such utilized markers were mentioned in Table 2. Appropriately the ISSR primers produced total of 148 repeatable and fixed bands (Table 3); of which 75 were polymorphic with a banding mean equal 14.67 for primers ISSR-2, ISSR-10, ISSR-11, ISSR-12, ISSR-15 and ISSR-18, consequently; while for the rest the mean of bands was 10.0. The size of bands as per molecular weights marker differed from 100 to 1517 bp (Figure 1).
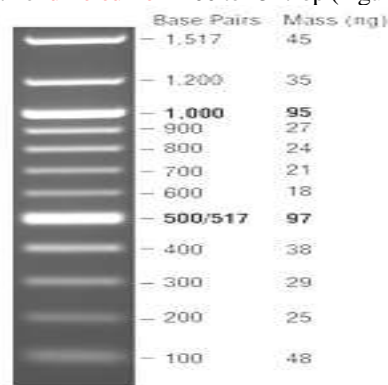
**Figure 1. Marker used for data analysis**

From past examinations it was discovered that three genotypes are tolerant to Bean yellow mosaic infection to be specific: Giza 843, X-2339 and Giza 716, respectively. Additionally, it was distinguished that four genotypes are somewhat tolerant to Bean yellow mosaic virus specifically: X-2338, X-2341, Sakha 1 and X-2342, respectively. In addition, it was discovered that three genotypes are powerless to Bean yellow mosaic infection in particular: X-2343, Sakha 4 and X-2340, respectively.

Concerning Giza 843 cultivar which show high tolerance to Bean yellow mosaic virus (El-Sayed *et al.*, 2019), It was discovered that Giza 843 had a unique band at 650 bp for ISSR-2; at 440 bp for ISSR-5; at 370 bp for ISSR-7; at 300 bp for ISSR-14 and at 300 bp for ISSR-15 (Figure 2 and Table 4) which might be utilized as a pointer for high resistance to Bean yellow mosaic infection. Simultaneously, nonappearance of some unique bands at 360 bp for ISSR-2 and at 360 bp for ISSR-12 may likewise be utilized as marker for the resistance to Bean yellow mosaic virus.

With respect to Sakha1 cultivar which uncovered resilience to Bean yellow mosaic infection (El-Sayed *et al.*, 2019), It was discovered that Giza 843 had an interesting unique bands at 460 bp for ISSR-5; at 430 bp for ISSR-6; at 330 bp for ISSR-6; at 730 bp for ISSR-11 and at 300 bp for ISSR-11, at 1200 bp for ISSR-14 and at 210 bp for ISSR-15 may use as an indicator for tolerance to Bean yellow mosaic virus (Figure 2 and Table 4).

Concerning Sakha4 cultivar which show high resistance to Bean yellow mosaic infection (El-Sayed *et al.*, 2019), It was discovered that Sakha 4 had unique band at 450 bp for ISSR-6; at 660 bp for ISSR-10; at 730 bp for ISSR-11; at 200 bp for ISSR-14 (Figure 2 and Table 4) which may answerable for high resilience to Bean yellow mosaic infection.
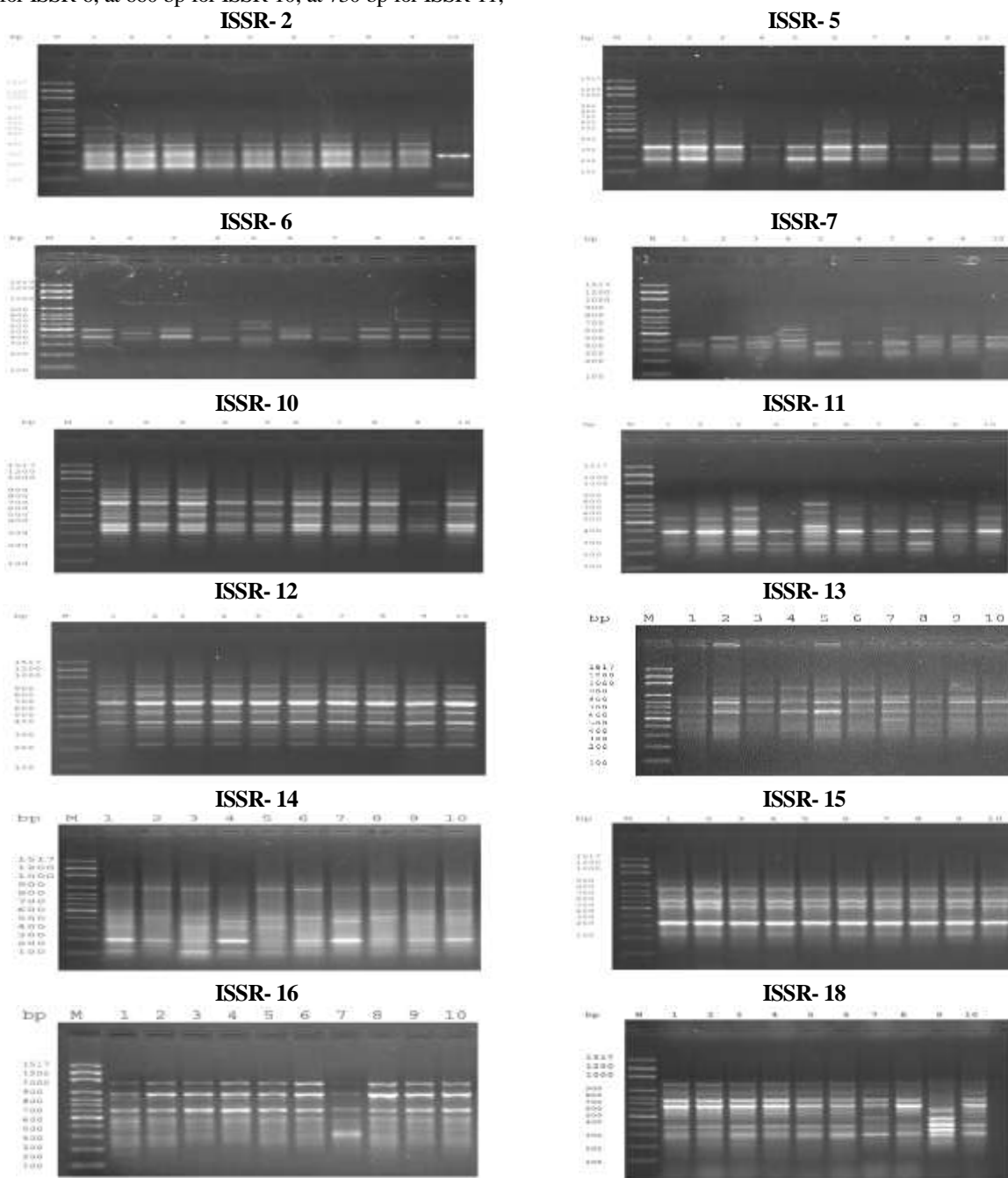
Figure 2. ISSR banding pattern generated by 12 primers of studying four faba bean cultivars and six promising genotypes. from left to right Giza 843, Sakha 1, Sakha 4, Giza 716, X-2338, X-2339, X-2340, X-2341, X-2342 and X-2343.

Table 3. ISSR Primers used in the study of four faba bean cultivars and six promising genotypes of faba bean, total number of fragments, number of polymorphic fragments, percent of polymorphism produced, common bands and unique fragments.

Gel Polymorphism													Total
ISSR primers	2	5	6	7	10	11	12	13	14	15	16	18	
Monomorphic bands	4	3	1	0	11	2	14	5	3	10	6	3	62
Polymorphic (without Unique)		4	7	10	7	10	3	4	8	3	4	9	75
Unique bands	3	0	3	1	0	1	0	0	0	0	1	2	11
Polymorphic (with Unique)	9	4	10	11	7	11	3	4	8	3	5	11	96
Total number of bands	13	7	11	11	18	13	17	9	11	13	11	14	148
Polymorphism (%)	69	57	91	100	39	85	18	44	73	23	45	79	50.67
Mean of band frequency	0.7	0.7	0.4	0.4	0.9	0.6	0.9	0.8	0.6	0.9	0.8	0.7	

Journal of Agricultural Chemistry and Biotechnology

Simultaneously, nonattendance of some unique bands at 470 bp and at 210 for ISSR-16 may likewise use as marker for the resilience to Bean yellow mosaic virus.

Regarding Giza 716 cultivar which show high tolerance to Bean yellow mosaic virus (El-Sayed *et al*., 2019), It was found that the cultivar Giza 716 had a unique band at 400 bp for ISSR-2; at 720 bp for ISSR-6; at 330 bp for ISSR-6; at 330 bp for ISSR-6, at 650, 560, 480, 370, 340

and 250 bp for ISSR-7 and at 1350 bp for ISSR-10 which may use as an indicator for high tolerance to Bean yellow mosaic virus. At the same time, absence of some unique bands at 460 bp for ISSR-2, at 230 bp for ISSR-5, at 430 and 200 bp for ISSR-10, at 320 bp for ISSR-11 and at 1000 bp for ISSR-13 may also use as indicator for the tolerance to Bean yellow mosaic virus (Figure 2 and Table 4)..

Table 4. Positive and negative, unique bands characterize the different types of tolerant genotypes depending on artificial infection with BYMV.

No.	Genotypes	Positive and negative unique DNA markers			Artificial infection	Indication
		+ or -	bp	Primer name		
1	Giza 843	+	650	ISSR-2	Mottle	High tolerant to BYV
2	Sakha 1	---	---	---	Mottle – Vein Yellowing	Tolerant
3	Sakha 4	+	450	ISSR-6	Mosaic - Deformation	Susceptible El-Sayed *et al*.,2019
4	Giza 716	-	460	ISSR-2	Mottle – Vein Yellowing	Less tolerance El-Sayed *et al*.,2019
		+	370	ISSR-7		
		-	340	ISSR-7		
		+	250	ISSR-7		
5	X-2338	+	320	ISSR-6	Mottle	Less tolerance El-Sayed *et al*.,2019
		+	280	ISSR-6		
		-	370	ISSR-16		
6	X-2339	---	---	---	Mottle – Vein Yellowing	Tolerant El-Sayed *et al*.,2019
7	X-2340	-	300	ISSR-16	Mosaic – Vein Yellowing - Deformation	Susceptible El-Sayed *et al*.,2019
8	X-2341	---	---	---	Mottle – Vein Yellowing	Less tolerant El-Sayed *et al*.,2019
9	X-2342	-	450	ISSR-10	Mottle – Vein Yellowing	Less tolerant El-Sayed *et al*.,2019
		-	210	ISSR-11		
		-	750	ISSR-11		
		+	500	ISSR-11		
		+	330	ISSR-18		
		+	1100	ISSR-18		
		-	800	ISSR-18		
		-	1000	ISSR-18		
10	X-2343	+	100	ISSR-21	Mosaic	Susceptible El-Sayed *et al*.,2019
		+	250	ISSR-21		
		-	260	ISSR-21		
		-	300	ISSR-21		
		-	530	ISSR-21		

Likewise the promising line X-2338, which revealed tolerance to Bean yellow mosaic virus (El-Sayed *et al*., 2019), It was found that X-2338 had a unique band at 440 bp for ISSR-5; at 320 bp and 280 bp for ISSR-6; at 180 bp for ISSR-7 and at 980, 700, 500 and 300 bp for ISSR-11.may use as an indicator for tolerance to Bean yellow mosaic virus. On the other hand, absence of some unique bands at 300 and 220 bp for ISSR-7, at 1350, 430 and 200 bp for ISSR-10, at 1000 bp for ISSR-13, and at 470 and 370 bp for ISSR-16 (Figure 2 and Table 4) may also use as indicator for the tolerance to Bean yellow mosaic virus.

Regarding the promising line X-2339, which revealed tolerance to Bean yellow mosaic virus (El-Sayed *et al*., 2019), It was found that X-2339 had a unique band at 460 bp for ISSR-5; at 430 bp for ISSR-6; at 220 bp for ISSR-7 and at 300 bp for ISSR-11.and at 300 and 200 bp for ISSR-14 may use as an indicator for tolerance to Bean yellow mosaic virus. On the other hand, absence of some unique bands at 300 bp for ISSR-7 and at 1000 bp for ISSR-13 (Figure 2 and Table 4) may also use as indicator for the tolerance to Bean yellow mosaic virus.

Concerning the promising line X-2340, which revealed tolerance to Bean yellow mosaic virus (El-Sayed *et*

al., 2019), It was found that X-2340 had a unique band at 330 and 430 bp for ISSR-6; at 650, 560, 480, 380 and 220 bp for ISSR-7 and at 200 bp for ISSR-14 may use as a marker for tolerance to Bean yellow mosaic virus. On the other hand, absence of some unique bands at 300 bp for ISSR-7, at 360 bp for ISSR-12, at 470, 310 and 210 bp for ISSR-16 and at 730 and 270 bp for ISSR-18 may also use as indicator for the tolerance to Bean yellow mosaic virus (Figure 2 and Table 4).

Regarding the promising line X-2341, which revealed tolerance to Bean yellow mosaic virus (El-Sayed *et al*., 2019), It was found that X-2341 had a unique band at 300 bp for ISSR-14 may use as an indicator for tolerance to Bean yellow mosaic virus. On the other hand, absence of some unique bands at 230 bp for ISSR-5, at 320 bp for ISSR-11, at 530 and 360 bp for ISSR-12 and at 730, 620, 540 and 270 bp for ISSR-18 (Figure 2 and Table 4) may also use as indicator for the tolerance to Bean yellow mosaic virus.

Concerning the promising line X-2342, which revealed tolerance to Bean yellow mosaic virus (El-Sayed *et al*., 2019), It was found that X-2342 had a unique band at 720 bp for ISSR-6; at 210 bp for ISSR-15 and at 1100 and 330 bp for ISSR-18 (Figure 2 and Table 4) may use as a marker for

* Corresponding author.
E-mail address: tamer.salem@fagr.bu.edu.eg

tolerance to Bean yellow mosaic virus. On the other hand, absence of some unique bands at 1350, 450 and 200 bp for ISSR-10, at 570, 320 and 220 bp for ISSR-11, at 1000, 800 and 300 bp for ISSR-18 may also use as indicator for the tolerance to Bean yellow mosaic virus.

In case of the promising line X-2343, which revealed tolerance to Bean yellow mosaic virus (El-Sayed *et al.*, 2019), It was found that X-2343 had a unique band at 645, 250 and 100 bp for ISSR-2; at 180 bp for ISSR-7 and at 730 bp for ISSR-11 may use as a marker for tolerance to Bean

yellow mosaic virus. On the other hand, absence of some unique bands at 530, 300 and 260 bp for ISSR-2, at 530 bp for ISSR-12, at 1200 bp for ISSR-14 and at 730 bp for ISSR-18 (Figure 2 and Table 4) may also use as indicator for the tolerance to Bean yellow mosaic virus.

Rp, PIC, and MI estimations uncovered that primer ISSR-14 (Figure 2), and (Table 5), can be considered proficient to analyze genetic variability with values of 5.8, 0.8, and 6.4, respectively, trailed by primers ISSR-6 and ISSR-18, which also showed efficiency in generating polymorphic amplicons.

Table 5. Values of Rp, PIC, and MI for each ISSR primer employed within these study.

	ISSR2	ISSR5	ISSR6	ISSR7	ISSR10	ISSR11	ISSR12	ISSR13	ISSR14	ISSR15	ISSR16	ISSR18
PIC	0.42	0.42	0.20	0.20	1.70	0.80	1.70	1.40	0.80	1.70	1.40	0.42
MI	3.78	1.68	2	2.2	11.9	8.8	5.1	5.6	6.4	5.1	7	4.62
Rp	2.2	2	4.8	5	3.8	5	1.8	3.2	5.8	2.6	1.8	4.4

Such results allow distinguishing that primers ISSR-6 and ISSR-7, amplified 7 and 10 total bands and for both 91 and 100% were polymorphic, (Terzopoulos *et al.* 2004 and Abdel-Razzak *et al.*, 2012) had been evaluated a similar (CA) 8G sequence in Greece and Egypt, consequently. By contrast, Terzopoulos and Bebeli (2008), Alghamdi *et al.* (2011), and Wang *et al.* (2012) assessing faba bean genotypes from Greece, Saudi Arabia, and China, separately, found higher polymorphism with primers of dinucleotide replications (AG) and (GA) anchored to mononucleotides and trinucleotides; sequences which were additionally distinguished in our materials but with less total bands. This proposes gene contrasts between the gathered material examined in this study and those stated for faba bean genotypes in other countries; such genetic diversity can be referred to the broad geographic separation between these materials. Probably the primers rich in (CA) may be tried with faba bean genotypes from other landmasses in order to confirm the gene likeness or distinction and recommend conceivable phylogenetic relations, according to Wang *et al.* (2012), faba bean genotypes belongs to China have their origin in gene sources from Africa, Asia and Europe, while there are no reports about this for genotypes from the American continent.

Genetic similarity between accessions:-

Dice's similarity coefficient has been used to calculate genetic relation between various faba bean genotypes via data analysis of ISSR markers. Genetic distances matrix illustrated an average rate from 0.80 to 0.91 (Table 3). Which indicates there is high DNA variability between accessions G9 and G10, both belong to the municipality of Egypt; this suggests they are genetically different materials adapted to the same environment, despite the fact that from a different origin. This can be favorable in projects of plant breeding, as it is commented by Gresta *et al.* (2010), utilization of this kind of materials might be used to boost the degree of variation and afterward evaluate these genotypes in contrasting environments that allow broadening their diversification. The longest distance value was registered between G2 and G6, which propose extraordinary genetic similarity; it can even be proposed that these populations originate from one same promotion, because of the nearness of the gathering sites and the portability of materials the growers make. Other very closely related accessions were: G1 and G2, G2 and G3, G3 and G6, G1 and G6. The rest of accessions displayed intermediate similarity levels.

Genetic variability between accessions: -

The genetic variability described in Table 3 was verified with UPGMA analysis (Figure 2). The dendrogram shows one main group and one accession (G9 or X-2342). The main group separated into two main sub-groups. The

first main subgroup includes two accessions; G4 (Giza 716) and G5 (X-2338). The second main subgroup include one main sub-group and one accession; G10 (X-2343). The second main sub-group was divided into two main sub-groups; the first main subgroup includes two accessions; G7 (X-2340) and G8 (X-2341).

The second main subgroup include one main sub-group and one accession; G1 (Giza 843). The last sub-group include one accession; G3 (Sakha 4) and sub-group consisted of two accessions G2 (Sakha2), and G6 (X-2339).

Table 6. Genetic distances matrix between the 10 faba bean genotypes, based on ISSR data and analyzed with Dice coefficient.

Genotypes	1	2	3	4	5	6	7	8	9	10
1	100									
2	90	100								
3	88	89	100							
4	83	82	82	100						
5	85	82	86	84	100					
6	88	91	89	85	87	100				
7	85	87	87	81	81	87	100			
8	88	86	87	82	81	85	87	100		
9	88	85	82	82	83	82	78	83	100	
10	86	86	86	80	81	82	84	87	80	100
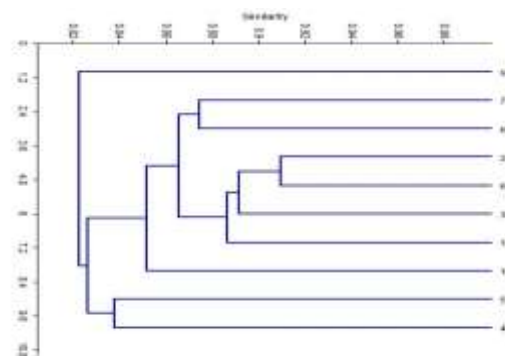
Figure 3. Cluster analysis based on ISSR markers for the study of genetic relationships among four faba bean cultivars and six promising faba bean genotypes.

These results agree with that reported by Terzopoulos and Bebeli (2008) and Wang *et al.* (2012), who declared that genetic diversity in faba bean is closely related to the environment in which it develops geographic root, and ecologic conveyance. Group II contain 60% of the genotypes of the central part, as well as two accessions from the north and south, G8 and G34, respectively. Genotype G8, from its grouping, shows that it is potentially a material with genes adaptive to agro-ecological differentiating conditions; these may be valuable to broaden the changeability degree. (Gresta *et al.*,

2010). Group V consists of five lines that belonged to the municipality of Acambay, found within the north; these genotypes are characterized by its little seeds and a precocious cycle. Whereas group VI was the biggest as contains 84% of the genotypes from the southeast study area; in this bunch the nearness of subgroups is discernable, likely from the intensive movement of the cultivators within this zone, since this is the biggest area to buy and sell seeds in the regions. The accessions in this group have a large seed and long cycle. Accessions G6, G7, and G9 from the north, and G26 and G31 were also part of this grouping. Similar results were found by Alghamdi *et al.* (2011), who mentioned that the existence of subgroups demonstrates genetic variability connected to acclimation to accumulation sites and that such distinctions may be utilized as gene sources to create lines for the development of synthetic varieties. By and large, the dendrogram permits deducing that groupings were decided somewhat by its geographic root, as well as by the genetic differences and similarities cumulated through time, sort of reproduction of faba beans, which are slightly allogamous, the fixed mobility of the materials the growers make and the facility of the species to adapt to different microenvironments.

Results of PCA Figure 2, and Table 5, uncover that the first three axes appear for only 33.99% of the overall variability; of these the two first coordinates cumulate 24.41% of variability and the first 13.45%. Furthermore, in this analysis three main groups (A, B, and C) and an unconventional one, comprising G7 (Figure 3), are observed. The dendrogram and PCA of the current investigation verified similarity between populations and variability was preserved; these results agree with that mentioned by Wang *et al.* (2012), who observed matching between both analyses, possibly because they worked with faba bean genotypes very distant geographically. It is worth mentioning that accessions G25 and G29, which appeared independent in the dendrogram, in PCA they grouped in B; whilst G37 and P38 in A. On its own, group C, held 80% of the northern populations. Distinguishable is that accession G7 unattached from the rest of populations (locating at -7.02, axis 2); a situation that was not observed in the dendrogram, this suggest it is a different material in molecular terms. This accession is cultivated at average altitudes of 2800 m (Table 1).

Finally, it is indicated that accessions G5, G6, G7, G8, G9, and G10 are molecularly different and can be assessed in the field to determine their agronomic potential. These accessions represent 60.00% of the total assessed material; this higher variability is considered enough to broaden the genetic base of the species in the Delta and Upper Egypt.

REFERENCES

Abdel-Razzak, H.S., A.M. Alfrmawy, H.M. Ibrahim, and A.A. El-Hanafy. 2012. Genetic diversity in faba bean (Vicia faba L.) using Inter-Simple Sequence Repeat (ISSR) markers and protein analysis. Life Science Journal, 9:497-503.

Alghamdi, S.S., S.A. Al Faifi, H.M. Migdadi, M.H. Ammar, and K.H.M. Siddique. 2011. Inter-simple sequence repeat (ISSR)-based diversity assessment among faba bean genotypes. Crop Pasture Science, 62(9):755-760.

Dellaporta, S.L., J. Wood, and J.B. Hicks. 1983. A plant DNA minipreparation: Version II. Plant Molecular Biology Reporter 1(4):19-20.

De Riek, J., E. Calsyn, I. Everaert, E. Van Bocksteal, and M. De Loose. 2001. AFLP based alternative for the assessment of the distinctness, uniformity and stability of sugar beet varieties. Theoretical and Applied Genetics 103:1254-1256.

El-Sayed1, A. F.; El-Shami, I.A. and Abeer M. Abo El-Wafa (2019): Susceptibility of some faba bean genotypes to bean yellow mosaic virus (BYMV) infection and the population of aphid vector (Aphis craccivora). Egypt. J. Plant Breed. 23(4):681– 699.

Gilbert, J.E., R.V. Lewis, M.J. Wilkinson, and P.S.D. Caligari. 1999. Developing and appropriate strategy to assess genetic variability in plant germplasm collections. Theoretical and Applied Genetics 98:1125-1131.

Gresta, F., G. Avola, E. Albertini, L. Raggi, and V. Abbate. 2010. A study of variability in the Silician faba bean landrace 'Larga di Leonforte'. Genetic Resources and Crop Evolution 57:523-531. doi:10.1007/s10722-009-9490-7.

Hoelzel, A.R. 1992. Molecular genetic analysis of populations: a practical approach. Oxford University Press, New York, USA.

Jones, R. A .C. (2005). Aphid-borne viruses in lupin stands under four different Patterns of spread of two non-persistently infection scenarios. Annals of Applied Biology 46:337- 357.

Jones, R.A.C. (2004). Using epidemiological information to develop effective integrated virus disease management strategies. Virus Res.100:5–30.

Khalil, S.A. and W. Erskine (2001). Combating disease problems of grain legumes in Egypt. Grain Legumes; a32 (2): 24-26.

Nei, M., and W.H. Li. 1979. Mathematical model for studying genetic variation in terms of restriction endonucleases. Proceedings of the National Academy of Sciences of the United States of America 76:5269-5273.

Pavlicek, A., S. Hrda, and J. Flegr. 1999. Free Tree-Freeware program for construction of phylogenetic trees on the basis of distance data and for bootstrap/jackknife analysis of the tree robustness. Application in the RAPD analysis of the genus Frenkelia. Folia Biology (Prague) 45:97-99.

Peakall, R., and P.E. Smouse. 2006. GENALEX 6: Genetic analysis in Excel. Population genetic software for teaching and research. Molecular Ecology Notes 6:288-295.

Peakall, R., and P.E. Smouse. 2012. GenAlEx 6.5: Genetic analysis in Excel. Population genetic software for teaching and research-an update. Bioinformatics 28:2537-2539.

Prevost, A., and M.J. Wilkinson. 1999. A new system of comparing PCR primers applied to ISSR fingerprinting of potato cultivars. Theoretical and Applied Genetics 98:107-112.

Roldán-Ruíz, I., J. Dendauw, E. Van Bockstaele, A. Depicker, and M. De Loose. 2000. AFLP markers reveal high polymhorphic rates in Ryegrasses (*Lolium spp*.) Molecular Breeding 6:125-134.

Terzopoulos, P.J., and P.J. Bebeli. 2008. Genetic diversity analysis of Mediterranean faba bean (*Vicia faba L.*) with ISSR markers. Field Crops Research 108(1):39-44.

Terzopoulos, P.J., P.J. Kaltsikes, and P.J. Bebeli. 2004. Characterization of Greek populations of faba bean (*Vicia faba* L.) and their evaluation using a new parameter. Genetic Resources and Crop Evolution 51:655-662.

Wang, H.F., X. Zong, J.P. Guan, T. Yang, X.L. Sun, Y. Ma, *et al*. 2012. Genetic diversity and relationship of global faba bean (*Vicia faba L.*) germplasm revealed by ISSR markers. Theoretical and Applied Genetics 124:789-797.

Yahia, Y., A. Guetat, W. Elfalleh, A. Ferchichi, H. Yahia, and M. Loumerem. 2012. Analysis of agromorphological diversity of southern Tunisia faba bean (Vicia faba L.) germplasm. African Journal of Biotechnology 11:11913-11924.

التقييم الجزيئي لعشرة طرز وراثية من الفول البلدى مختلفة في قابلية الإصابة بفيروس التبرقش الأصفر

تامر محمد شحاتة سالم*

قسم الوراثة والهندسة الوراثية ، كلية الزراعة ، جامعة بنها ، القليوبية 13511، جمهورية مصر العربية

الهدف من هذه الدراسة هو تحديد درجة التباين الوراثي وكذلك تحليل العلاقة بين عشرة طرز وراثية من الفول البلدى عبارة عن أربعة أصناف منزرعة بالإضافة إلى ستة سلالات مبشرة وذلك بتحديد كشافات وراثية جزيئية بإستخدام تقنية التتابعات البسيطة المتسلسلة (ISSR) . تم إختيار العشرة تراكيب وراثية من الفول البلدى التى تم إستخدامها على حسب درجة مقاومتها لفيروس التبرقش الأصفر للفول البلدى. مع تسجيل نطاق الحزم التي تم الحصول عليها ، تم بناء مصفوفة بيانات ثنائية لإجراء التحليل الإحصائي المقابل. أنتجت علامات ISSR المستخدمة 148 حزمة بشكل ثابت ومتكرر لكل الطرز الوراثية 75 حزمة متباينة التواجد بالنسبة للتركيب الوراثى. كشفت قيم قدرة الاستبانة (Rp) ، ومحتوى معلومات الأشكال المتعددة (PIC) ، ومؤشر العلامة (MI) ، على التوالي أن البادىء14-ISSRكان الأكثر فعالية في تحليل التباين الوراثي بقيم 5,8 و 0,80 و 6,4 على التوالي ، متبوعا بالبوادىء ISSR-6 ثم ISSR-18 . تذبذبت المسافات الجينية بين 0,78 و 0,91 ، بالتحقق من التجمعات التي لوحظت في شجرة التفريع (الدندروجرام)، تم الإستدلال على زيادة التباين على مستوى الحمض النووي بين الأنماط الوراثية التي تم تحليلها ، مع ملاحظة ست مجموعات محددة وفقا لتحليل UPGMA في تحليل المكونات الرئيسية. كما تم تحديد التجمعات المسجلة حسب أصل كل تجمع. توصى هذة الدراسة بأن إستخدام كشافات وراثية جزيئية بتقنية الـ ISSR يعتبر إجراء فعالاً لتوصيف التراكيب الوراثية المستخدمة على مستوى الحمض النووي ، كما يمكننا من إستخدام التراكيب الوراثية المتباينة في برامج التحسين الوراثي للفول البلدى التي تهدف إلى تلبية احتياجات المنتج والمستهلك.