

IDENTIFICATION OF SOME FABA BEAN (*Vicia faba* L.) GENOTYPES USING MORPHOLOGICAL AND MOLECULAR CHARACTERS

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ABSTRACT

Field and laboratory experiments were carried out at the Farm of El-Gemmeza Agricultural Research Station, Gharbia Governorate and Seed Technology Research Department, ARC, Egypt, during 2010/2011 and 2011/2012 seasons to identify and discriminate ten faba bean genotypes using morphological characters and molecular marker. The results revealed that some morphological characters such as pinnul shape, lines density of flag flower, pod color at maturity, testa shape and color were useful to identify some genotypes from each other, while they were not enough for identifying other genotypes. By using Inter-simple sequence repeat (ISSR-PCR) technique, it was possible to determine the genetic diversity and relationships of the ten faba bean genotypes included in this study. A total of 71 amplified bands were generated with five ISSR primers, of which 59 (83.1%) were polymorphic which represent a relatively high polymorphism level. These results are important in protecting of plant breeders rights and at releasing these genotypes as a new varieties.

INTRODUCTION

The morphological, quantitative and biochemical characters study was designed to find out distinguished characters of faba bean genotypes. Morphological characterization is the first step in the classification and description of any crop germplasm. Nevertheless, the qualitative traits are often used for separating varieties when a limited range of quantitative traits are found in certain group. Furthermore, morphological description is a precondition for the protection and registration of varieties (UPOV 2002). Germplasm evaluation is considered the first step in plant breeding program and it is commonly based on a simultaneous examination of large number of populations for several characters of both agronomic and physiological interest (Pezzotti *et. al.* 1994). Cultivars within species are normally discriminated by morphological descriptors. Many tools are now available for studying genetic variability among accessions including total seed protein, isozymes and various types of molecular markers. Rehab, Tawdy (2007), identified ten vicia faba varieties based on morphological differences in seed, seedling and adult plant such as days from planting to flowering and maturity, anthocyanin coloration, color of testa, number of pods and 1000 seed weight), in addition to biochemical variability of genomic fingerprinting. Zubair *et. al.*, (2007) evaluated 14 quantitative traits for forty diverse mungbean [*Vigna radiata* (L.) Wilczek] genotypes, they found that medium to high variance was observed for days to flower initiation, days to flowering, days to maturity, plant height and pods per plant. Meanwhile, small variance was observed for seeds per pod and 100 seed weight. Lalazar (2012) evaluate

the diversity of phenology and morphology traits such as days from germination to flowering and maturity, flower bed length, flowers number and length, flower pedanlel length and 1000 seed weight in 11 genotypes, he reported that the highest days from germination to flowering was in Potomak and Harvester genotypes and the lowest in Wadekh genotype (31 day). The highest number of pod was in Potomak and Harvester genotypes and the lowest in Saksa b/v 615 and Oltin genotypes. The highest 1000 seed weight was noticed from Potomak genotype and same genotype had the lowest days from germination to maturity (50 day). Mudzana *et al.* (1995) reported that the morphological characters such as plant height, number of days to 50 per cent flowering, flower length, pod length, number of seeds per pod could be used for variety identification of faba beans. Bonetti *et al.* (1995) reported that 17 bean cultivars were grouped based on pod length (very short, short, medium, long, very long), maturity (early, medium, late) and time of flowering (early, medium and late). Ashok *et al.* (2008) grouped seven french bean varieties based on hilum color and seed shape. On the other hand, the most commonly used polymerasechain reaction (PCR)-based marker systems for genetic diversity and relationships in faba bean species are randomly amplified polymorphic DNA (RAPD) (Link *et al.*, 1995), amplified fragment length polymorphism (AFLP) (Duc *et al.*, 2010) and species specific repeats (SSR) (Zeid *et al.*, 2009). The main limitations of these methods are low reproducibility of RAPD, high cost of AFLP and the necessity to know the flanking sequences to develop species specific primers for SSR polymorphism (Belaj *et al.*, 2003; Jabbarzadeh, *et al.*, 2010). Inter-simple sequence repeat (ISSR-PCR) is a route that overcomes most of these technical limitations (Chen *et al.*, 2008). ISSR markers have been widely applied to characterize plant germplasm and to demonstrate its effectiveness in assessments of plant genetic diversity (Galvain *et al.*, 2003, Pharmawati *et al.*, 2005 and Bhagyawant and Srivastava 2008).

The purpose of the present study was to identify ten faba bean genotypes by using some morphological traits and describe the genetic diversity of these by using Inter-Simple Sequence Repeats (ISSRs) and classify these genotypes.

MATERIALS AND METHODS

Field and laboratory experiments were carried out at Gemmeiza Agriculture Research Station, Gharbia Governorate, and Seed Technology Research Department, ARC, Giza during 2010/2011 and 2011/2012 seasons. Seeds of the studied genotypes were received from Legume Research Dep., Field Crops Research Institute, Agricultural Research Center. The experimental design was a Randomized Complete Block design with three replications. Seeds were inoculated and hand planted. Sowing date was 15th November in the first season, while it was 20th November in the second season. All the agronomic practices were conducted as recommended and the studied traits were as follows:-

Morphological characters

Qualitative traits were visually recorded using scales reported by IBPGR (1984). These characters included; anthocyanin coloration, leaf size spots on ears, pinnul shape, color of stem, color of flower ground, lines density on flag flower, wing color of flower, pod corner with stem, pod shape, reversal of pod surface, pod color at maturity, pods division on stem, color of testa, color of Hilum, seed shape and testa shape.

Quantitative characters

These characters included; days from sowing to 50% flowering of plants with at least one flower and days from sowing to maturity, stem thickness, time of beginning of flowering, time of maturity, highest of the first pod (cm), number of pinnule/ leaf, branching grassroots, number of flowers/ marble, number of pod at nods, number of seeds/ pod, plat height (cm) and seed index. Collected data for each season were statistically analyzed and the Least Significant Difference (L.S.D.) was used to compare among them (Gomez and Gomez, 1984).

Molecular markers

Plant material and DNA extraction:

DNA was extracted from the tissue of young, healthy leaf which was selected from each genotype, using the DNA extraction kit (Quigen Inc., Cat.no.69104, USA). DNA quality was tested using 1% agarose gel electrophoresis and its concentration was determined spectrophotometrically.

ISSR-PCR analyses

Five ISSR primers were selected for testing the genetic diversity between these genotypes. Names and sequences of these primers are shown in Table (1). The PCR reaction was carried out in a 25 µl volume of a mixture containing 25 ng of genomic DNA, 0.1 mM dNTPs, 2.5 mM MgCl₂, 1 unit Taq polymerase, 10x Taq buffer and 0.6 µM primer. DNA amplification was carried out using the thermocycler model PTC 200 (MJ Research, Watertown, MA, USA). The amplification program included a denaturing step at 94 °C for 5 min, followed by 35 cycles with a denaturing step at 94 °C for 1 min, an annealing step at 53 °C for 1 min and an extension step at 72 °C for 2 min. After the last cycle, samples were kept at 72 °C for 5 min.

Gel electrophoresis

Gel electrophoresis was applied according to Sambrook *et al.* (1989). Agarose (1.2 %) was used for resolving the PCR products. Bands were detected on UV-transilluminator and photographed by Gel documentation 2000, Bio- Rad. Similarity and dendrogram tree was performed using the SPSS program version 10.

Table (1): Names and sequences of the five primers used for ISSR-PCR analyses.

Primer name	Sequence
SH6	5' CGCGATAGATAGATAGATA 3'
SH7	5' GACGATAGATAGATAGATA 3'
SH8	5' AGACAGACAGACAGACGC 3'
SH9	5' GATAGATAGATAGATAGC 3'
SH10	5' GACAGACAGACAGACAAT 3'

RESULTS AND DISCUSSION

Some morphological characters of the different faba bean genotypes are presented in Table (2). The genotypes ($G_{429} \times G_{40}$) and ($H_8 \times G_{461}$) identified with anthocyanin coloration mixture and mixture violet, respectively while in the other faba bean genotypes it was absent, the genotypes ($G_{Blanka} \times G_2$) and ($H_{10} \times G_{461}$) have weak violet.

Table (2): Some morphological characters of faba bean genotypes (combined data of 2010/ 2011 and 2011/2012 growing seasons).

Characters Genotypes	Anthocyanin coloration	Leaf size	Spots on ears	Pinnul shape	Color of stem	Color of flower ground
$G_{461} \times G_{Blanka}$	Absent	Large	Present	Semi Flat	Light brown	Light brown
T.W. x G_{Blanka}	Absent	Medium	Absent	Round	Brown	Light brown
$G_{716} \times G_{402}$	Absent	Small	Present	Round	Brown	Light brown
$G_{461} \times G_{402}$	Absent	Medium	Present	Round	Light brown	White
$G_{Blanka} \times G_2$	Weak Violet	Small	Absent	Narrow	Brown	White
$G_{429} \times G_2$	Absent	Medium	Present	Narrow	Light brown	White
$G_{429} \times G_{40}$	Mixture	small	Present	Narrow	Brown	White
$H_8 \times G_{461}$	Medium violet	Large	Absent	Narrow	Very light brown	White
$H_{10} \times G_{461}$	Weak Violet	Medium	Present	Round	Light brown	White
T.W. $G_{461} \times Egypt_1$	Absent	Large	Present	Round	Dark brown	White

Leaf size can divide the tested genotypes into three groups; small ($G_{716} \times G_{402}$, $G_{Blanka} \times G_2$, and $G_{429} \times G_{40}$), medium (T.W.x G_{Blanka} , $G_{461} \times G_{402}$ and $H_{10} \times G_{461}$) and large ($G_{461} \times G_{Blanka}$, $H_8 \times G_{461}$, and (T.W.x G_{461})xEgypt₁). Regarding Spots on ears, they were present in the genotypes ($G_{461} \times G_{Blanka}$, $G_{716} \times G_{402}$, $G_{461} \times G_{402}$, $G_{429} \times G_2$, $G_{429} \times G_{40}$, $H_{10} \times G_{461}$ and (T.W.x G_{461})xEgypt₁ and absent in the genotypes (T.W.x G_{Blanka} , $G_{Blanka} \times G_2$, and $H_8 \times G_{461}$). The genotype ($G_{461} \times G_{Blanka}$) identified with pinnul shape (semi flat). The genotypes ($H_8 \times G_{461}$ and (T.W.x G_{461})xEgypt₁) were identified with color of stem (very light brown and dark brown), respectively. Color of flower ground characters divided faba bean genotypes under studied into two classes, the first class, concluded the genotypes ($G_{461} \times G_{402}$, $G_{461} \times G_{Blanka}$, T.W.x G_{Blanka} and $G_{716} \times G_{402}$) light brown. Meanwhile, the second class (white) was concluded the genotypes ($G_{461} \times G_{402}$, $G_{Blanka} \times G_2$, $G_{429} \times G_2$, $G_{429} \times G_{40}$, $H_8 \times G_{461}$, $H_{10} \times G_{461}$ and (T.W.x G_{461})xEgypt₁). These results are in agreement with Rehab, Towdy (2007) she reported that, anthocyanin coloration was absent in Sahel 1 while in the other varieties was present, Intensity of anthocyanine coloration was medium in Mesr 1 but was slight for the rest of

varieties and showed that, Mesr 1 and Nubaria 1 varieties were short in leaflet size while the other varieties were medium.

Data in Table (3), show some morphological characters of different faba bean genotypes included in this study. The genotype ($G_{461} \times G_{Blanka}$) identify with lines density on flag flower (dense). Regarding wing color of flower, all faba bean genotypes were black spot. Pod corner with stem can divide the tested genotypes into two groups; the genotypes ($G_{461} \times G_{Blanka}$, $G_{429} \times G_2$, $G_{429} \times G_{40}$, $H_8 \times G_{461}$ and $(T.W. \times G_{461}) \times Egypt_1$) were mixture and the genotypes ($T.W. \times G_{Blanka}$, $G_{716} \times G_{402}$, $G_{461} \times G_{402}$, $G_{Blanka} \times G_2$ and $H_{10} \times G_{461}$) were existing. The genotypes under study divided into three class according to pod shape the genotypes ($G_{461} \times G_{Blanka}$, $T.W. \times G_{Blanka}$, $G_{716} \times G_{402}$, $G_{429} \times G_2$, $G_{429} \times G_{40}$ and $H_8 \times G_{461}$) have semi-cylindrical while, the genotypes ($G_{461} \times G_{402}$ and $(T.W. \times G_{461}) \times Egypt_1$) were narrow extrovert and the genotypes ($G_{Blanka} \times G_2$ and $H_{10} \times G_{461}$) were extrovert non narrow under pod shape. Regarding reversal of pod surface, the genotypes ($G_{461} \times G_{Blanka}$, $G_{429} \times G_2$, $G_{429} \times G_{40}$ and $(T.W. \times G_{461}) \times Egypt_1$) have shiny surface and the genotypes ($T.W. \times G_{Blanka}$, $G_{716} \times G_{402}$, $G_{461} \times G_{402}$, $G_{Blanka} \times G_2$, $H_8 \times G_{461}$ and $H_{10} \times G_{461}$) have matted pod surface. The genotypes ($G_{461} \times G_{Blanka}$, $G_{429} \times G_2$, $G_{429} \times G_{40}$, $H_8 \times G_{461}$ and $(T.W. \times G_{461}) \times Egypt_1$) can identified with pod color at maturity Dark black, Black, Mixed, White yellow and White brown respectively, while other genotypes were brown.

Table (3): Some morphological characters of faba bean genotypes (combined data of 2010/ 2011 and 2011/2012 growing seasons).

Characters Genotypes	Lines density on flag flower	Wing color of flower	Pod corner with stem	Pod shape	Reversal of pod surface	Pod color at maturity
$G_{461} \times G_{Blanka}$	Dense	Black spot	Mixture	Semi-cylindrical	Shiny	Dark black
$T.W. \times G_{Blanka}$	Medium	Black spot	Existing	Semi-cylindrical	Matted	Brown
$G_{716} \times G_{402}$	Slight	Black spot	Existing	Semi-cylindrical	Matted	Brown
$G_{461} \times G_{402}$	Slight	Black spot	Existing	Narrow extrovert	Matted	Brown
$G_{Blanka} \times G_2$	Slight	Black spot	Existing	Extrovert non narrow	Matted	Brown
$G_{429} \times G_2$	Medium	Black spot	Mixture	Semi-cylindrical	Shiny	Black
$G_{429} \times G_{40}$	Slight	Black spot	Mixture	Semi-cylindrical	Shiny	Mixed
$H_8 \times G_{461}$	Medium	Black spot	Mixture	Semi-cylindrical	Matted	White yellow
$H_{10} \times G_{461}$	Medium	Black spot	Existing	Extrovert non narrow	Matted	Brown
$T.W. \times G_{461} \times Egypt_1$	Slight	Black spot	Mixture	Narrow extrovert	Shiny	White brown

Also some morphological characters of the studied faba bean genotypes are given in Table (4). It is clear that, pods division on stem divided the genotypes under studied into two groups, the genotypes ($G_{461} \times G_{Blanka}$, $T.W. \times G_{Blanka}$, $G_{716} \times G_{402}$ and $G_{461} \times G_{402}$) were both sides of meanwhile, the other genotypes were homogeneous. The genotypes ($T.W. \times G_{Blanka}$ and $H_{10} \times G_{461}$) identified with color of testa (White yellow and yellow, respectively). Color of hilum of all genotypes under study was black. Regarding seed shape, the genotypes ($G_{461} \times G_{Blanka}$, $T.W. \times G_{Blanka}$, $G_{716} \times G_{402}$, $G_{Blanka} \times G_2$, $H_8 \times G_{461}$, $H_{10} \times G_{461}$ and $(T.W. \times G_{461}) \times Egypt_1$) have extrovert and the genotypes ($G_{461} \times G_{402}$, $G_{429} \times G_2$ and $G_{429} \times G_{40}$) have mixture shape. The genotype ($G_{429} \times G_{40}$) identity with testa shape (small patches) while, the other genotypes were fate. These results consent with Rehab tawdy (2007), she found that color of testa was beige in all the varieties of faba bean under studies except for G.717 which was semi beige green.

Table (4): Some morphological characters of faba bean genotypes (combined data of 2010/2011 and 2011/2012 growing seasons).

Characters Genotypes	Pods division on stem	Color of testa	Color of Hilum	Seed shape	Testa shape
$G_{461} \times G_{Blanka}$	Both sides of	Dark green	Black	Extrovert	Fate
$T.W. \times G_{Blanka}$	Both sides of	White yellow	Black	Extrovert	Fate
$G_{716} \times G_{402}$	Both sides of	Wight green	Black	Extrovert	Fate
$G_{461} \times G_{402}$	Both sides of	Mixed	Black	Mixture	Fate
$G_{Blanka} \times G_2$	Homogeneous	Wight brown	Black	Extrovert	Fate
$G_{429} \times G_2$	Homogeneous	Wight brown	Black	Mixture	Fate
$G_{429} \times G_{40}$	Homogeneous	Mixed	Black	Mixture	small patches
$H_8 \times G_{461}$	Homogeneous	Wight brown	Black	Extrovert	Fate
$H_{10} \times G_{461}$	Homogeneous	Yellow	Black	Extrovert	Fate
$T.W. \times G_{461} \times Egypt_1$	Homogeneous	Dark green	Black	Extrovert	Fate

Combined data of the quantitative characters of the studied faba bean genotypes are presented in Tables (5 and 6). It is clear that, two characters namely height of the first nod and time of beginning of flowering were to some extent effective tools to differentiate between genotypes. Whereas as studied characters were almost similar. The genotype ($H_8 \times G_{461}$) gave the highest stem thickness (0.9 cm) and seed index (89.5 gm). The genotype ($G_{429} \times G_{40}$) recorded maximum of the first nod (31.1 cm) while, the genotype ($H_8 \times G_{461}$) recorded the lowest height of the first nod (24.5 cm). The genotype ($G_{429} \times G_2$) recorded the tallest plant height (102.5 Cm) on the other hand, the genotype ($G_{429} \times G_{40}$) recorded the shortest plant height (96.0 cm). Tallest pod length was noticed by the genotype ($G_{Blanka} \times G_2$) 9.9 cm while,

shortest pod length was noticed by (G₄₆₁xG.Blanka). Concerning time of beginning of flowering and maturity the genotype (T.W.G₄₆₁xEgypt₁) was the latest among all the genotypes (55 and 156 days from begging planting) respectively, while the genotype (H₁₀ x G₄₆₁) was the earliest for beginning of flowering and maturity 46 and 152 days from begging planting, respectively comparing with the other genotypes. The genotypes (G.Blanka x G₂ and H₁₀ x G₄₆₁) recorded highest No. of pinnule/ leaf (5 pinnule) but, the genotype (G₄₆₁ x G₄₀₂) have little No. of flowers/ marble (3 flowers) comparing with the other genotypes. No. of seeds/ pod was (4 seeds) at (G₄₂₉ x G₄₀ and T.W.G₄₆₁xEgypt₁) genotypes, while it was (3 seeds) at all the other genotypes. Similar results were in agreement with Ibrahim (2010) and Al Barri and Munqez (2013), they revealed that VF-14, VF-10 and VF-12 lines significantly longer days to flowering and differed from all the other genotypes, VF-8, VF-12 and VF-13 lines were significantly the tallest plants while, VF-6 and VF-7 were the shortest, VF-11 line showed the lowest average of pod height and VF-19 gave the highest value, VF-4 and VF-10 lines showed the highest pod length, while VF-2 genotype gave the lowest pod length, VF-10 line significantly had the highest average seed number per pod while VF-7 and VF-19 significantly showed the lowest average seed number per pod and VF-4 genotype gave significantly the highest 100-seed weight while, VF-17 and VF-2 gave the lowest 100-seed weight.

Table (5): Some quantitative characters of faba bean genotypes (combined data of 2010/ 2011 and 2011/2012).

Characters Genotypes	Stem thickness (cm)	Height of the first nods (cm)	Time of beginning of flowering	Time of maturity	No. of pinnule/ leaf
G ₄₆₁ xG.Blanka	0.8	26.5	48	154	4
T.W.xG.Blanka	0.8	29.0	47	154	4
G ₇₁₆ x G ₄₀₂	0.7	25.5	49	155	4
G ₄₆₁ x G ₄₀₂	0.8	26.6	48	153	4
G.Blanka x G ₂	0.8	29.4	50	154	5
G ₄₂₉ x G ₂	0.8	26.9	47	153	4
G ₄₂₉ x G ₄₀	0.8	31.1	47	153	4
H ₈ x G ₄₆₁	0.9	24.5	50	153	4
H ₁₀ x G ₄₆₁	0.8	26.6	46	152	5
T.W.G ₄₆₁ xEgypt ₁	0.7	29.1	55	156	4
L.S.D. at 5%	0.1	2.7	0.95	1.45	1.0

Table (6): Some quantitative characters of faba bean genotypes (combined data of 2010/ 2011 and 2011/2012).

Characters Genotypes	No. of flowers/ marble	No. of pods at nods	No. of seeds/ pod	Pod length (cm)	Plant height (cm)	Seed index (gm)
G ₄₆₁ x G.Blanka	4	2	3	8.6	100.3	83.8
T.W.xG.Blanka	4	2	3	9.4	99.6	87.5
G ₇₁₆ x G ₄₀₂	4	1	3	9.2	98.6	79.5
G ₄₆₁ x G ₄₀₂	3	2	3	9.1	102.0	87.2
G.Blanka x G ₂	5	2	3	9.9	96.8	83.6
G ₄₂₉ x G ₂	4	1	3	9.0	102.5	83.8
G ₄₂₉ x G ₄₀	5	1	4	9.5	96.0	82.3
H ₈ x G ₄₆₁	5	2	3	8.9	101.5	89.5
H ₁₀ x G ₄₆₁	5	2	3	9.0	98.0	87.7
T.W.G ₄₆₁ x Egypt ₁	4	1	4	8.9	96.3	85.2
L.S.D. at 5%	1.0	1.0	1.0	0.6	4.7	1.9

Molecular marker

In the present study, five selected primers of ISSR were used to differentiate between the ten faba bean genotypes (Figure 1). Inter-Simple Sequence Repeat (ISSR) technique yield more polymorphisms than other molecular techniques. The five primers amplified different numbers of bands and revealed various levels of polymorphism. A total of 71 ISSR loci were observed and 59 (83.1%) of them were polymorphic. these primers yielded 14, 11, 18, 14 and 14 bands, respectively (Table 7). The percentage of polymorphism was 92.9 %, 90.9 %, 72.2 %, 85.7 % and 78.6 %, respectively. The primer SH8 yielded the largest number of bands (18 band) while, primer SH7 had fewer number of bands. Among all the ISSR loci observed, 11 were unique. The highest number of unique band can observed in primer SH8 which produced four markers. While, the lowest number can observed in primer SH7 and SH10 which produced one marker. The ISSR primer method is reported to produce more complex marker (Parsons *et. al.*, 1997 and Chowdhury *et. al.*, 2002), which is advantageous when differentiating closely related cultivars.

Table (7): Levels of polymorphism and unique genotypes specific bands for ten faba bean genotypes by five ISSR primers.

Bands Primer	Total bands	Polymorphic bands	Monomorphic bands	Polymorphism %	Unique bands	
					Genotypes	Ms (bp)
SH6	14	13	1	92.9	3	938
					5	341
					9	228
SH7	11	10	1	90.9	3	800
					9	1493
SH8	18	13	5	72.7	8	927
					3	644
					6	266
					5	681
SH9	14	12	2	85.7	4	556
					2	729
SH10	14	11	3	78.6	2	729
Total	71	59	12	83.1		

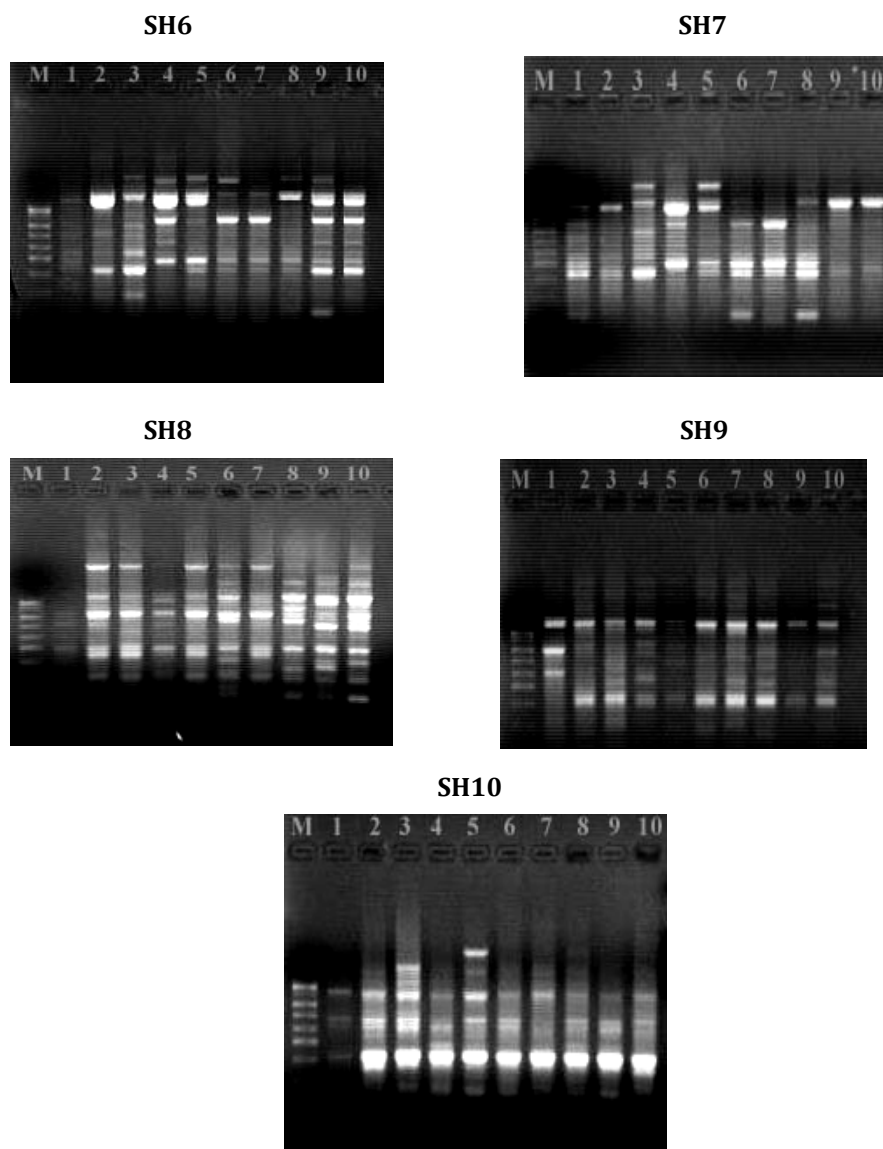


Figure (1): ISSR fingerprinting of ten faba bean genotypes, Lines from left to right: M= Marker, G₄₆₁XG_{Blanka}, T.W.XG_{Blanka}, G₇₁₆XG₄₀₂, G₄₆₁XG₄₀₂, G_{Blanka}XG₂, G₄₂₉XG₂, G₄₂₉XG₄₀, H₈XG₄₆₁, H₁₀XG₄₆₁ and (T.W.x G₄₆₁)x Egypt₁.

Genetic diversity

Standard genetic distances could be estimated between all the genotypes showed in Table (8). The genetic similarity ranged from 81% to 53% with an average of 67%. The closest distance was found between the genotypes (G₄₂₉XG₂) and (T.W.x G₄₆₁)x Egypt₁) and also between (H₁₀XG₄₆₁)

and (T.W.x G₄₆₁)x Egypt₁ (81%), while the longest was between the genotypes (G₇₁₆xG₄₀₂) and (H₁₀xG₄₆₁) (53%). The results of clustering genetic distance using the unweighted pair group method separated genotypes to two main clusters with many subclusters, where, the genotype1 (G₄₆₁xG_{Blanka}) was in a separate subcluster, while, the other genotypes were in the second subcluster (Figure 2). Salem *et. al.*, (2011) subjected Thirty-four faba bean (*Vicia faba* L.) to molecular diversity assessment using 12 inter-simple sequence repeat primers and found that there is a high genetic variability related to collection sites and it should be utilised in faba bean improvement. Also, Abdel-Razzak *et. al.*, (2012) clarified that ISSR markers and protein analysis were helpful to recognize genetic variation among faba bean cultivars.

Table (8): Similarity matrix among ten faba bean genotypes based on ISSR analysis.

Genotypes	G ₄₆₁ X G _{Blanka}	T.W.x G _{Blanka}	G ₇₁₆ X G ₄₀₂	G ₄₆₁ X G ₄₀₂	G _{Blanka} X G ₂	G ₄₂₉ X G ₂	G ₄₂₉ X G ₄₀	H ₈ X G ₄₆₁	H ₁₀ X G ₄₆₁
G ₄₆₁ XG _{Blanka}									
T.W.x G _{Blanka}	0.64								
G ₇₁₆ XG ₄₀₂	0.64	0.74							
G ₄₆₁ XG ₄₀₂	0.63	0.60	0.61						
G _{Blanka} XG ₂	0.56	0.69	0.63	0.62					
G ₄₂₉ XG ₂	0.70	0.64	0.61	0.71	0.72				
G ₄₂₉ XG ₄₀	0.69	0.74	0.73	0.70	0.68	0.77			
H ₈ XG ₄₆₁	0.65	0.68	0.72	0.69	0.69	0.78	0.79		
H ₁₀ XG ₄₆₁	0.60	0.61	0.53	0.69	0.66	0.73	0.60	0.68	
(T.W.x G ₄₆₁)x Egypt ₁	0.66	0.74	0.61	0.70	0.68	0.81	0.76	0.72	0.81

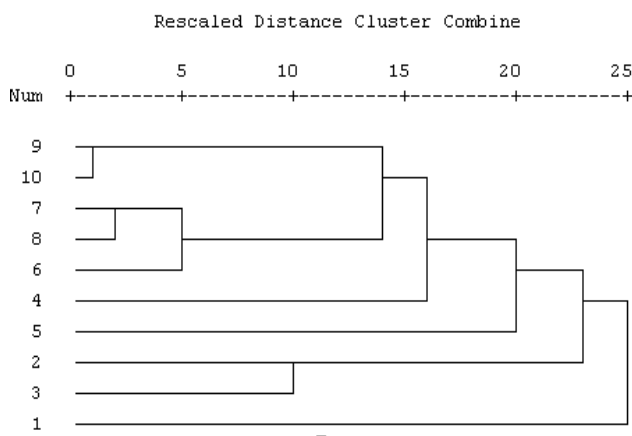


Figure (2): Dendrogram of the genetic distances among the ten faba bean genotypes based on ISSR analysis.

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التمييز المورفولوجي والجزئي لبعض التراكيب الوراثية من الفول البلدي
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تهدف هذه الدراسة الى استخدام بعض الصفات المورفولوجية والجزئية لتمييز عشرة تراكيب وراثية من الفول البلدي من خلال إجراء بعض التجارب الحقلية والمعملية بمحطة البحوث الزراعية بالجميزة ومعامل قسم بحوث تكنولوجيا البذور بالجميزة – مركز البحوث الزراعية، خلال موسمي 2011/2010، 2012/2011 م. وأوضحت النتائج المتحصل عليها أن بعض الصفات المورفولوجية مثل شكل الوريقة الوسطى، كثافة الشرائط (الخطوط) على علم الزهرة، لون القرن عند النضج وشكل ولون قصرة البذرة كانت وسيلة فعالة لتمييز بعض التراكيب الوراثية بينما لم تكن فعالة لتمييز بعض التراكيب الوراثية الأخرى، كما أمكن تمييز الاختلافات الوراثية بين التراكيب الوراثية التي اشتملت عليها الدراسة على المستوى الجزئي وذلك باستخدام خمسة من البادئات العشوائية وتكنيك ISSR-PCR. وقد بلغت نسبة التشابه من (53-81%) بمتوسط قدرة (67%). والنتائج المتحصل عليها من هذه الدراسة ذات أهمية كبيرة في حفظ حقوق مربو النباتات عند تسجيل التراكيب الوراثية كأصناف تجارية جديدة إلا أنه علي مربو النبات الانتحاب من قاعدة وراثية عريضة حتى يمكن الحصول علي صفات مورفولوجية مميزة للسلاسل الجديدة عن الأصناف المنزرعة المسجلة وذلك عند تسجيلها كأصناف جديدة مما يسهل التحقق من نقاوة الصنف الجديد أثناء مراحل اكثاره المختلفة.