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Estimation of genetic variation of Faba bean genotypes using agronomic traits, biochemical, ISSRs and SCOTs markers

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BREEDING programs with estimating of genetic variation are important to obtain the best results. In present study, we have evaluated eighteen faba bean genotypes for earliness, yield and yield attributes and assessed genetic diversity using protein, ISSR and SCoT marker. High significant differences were observed between genotypes for earliness traits, yield and its attributes in both seasons. Also, the yield results and its attributed pointed out that the highest values of these traits were produced by G.3, 114, 220, 212, 180 A and 180 C genotypes in two seasons. Meanwhile, we recorded the lowest values of these characters in 171, 168 and G2 genotypes. On the other side, the correlation coefficients were significant between number of pods/nod and each number of seeds/pod, number of seeds/plant, 100-seed weight and seed weight/plant in both seasons, respectively. Seven ISSR and six SCoT primers had success to produce polymorphic bands that were effective for studying the genetic variation between the studied genotypes. Out of 149 clear bands detected, 57 for ISSR and 76 for SCoT were polymorphic with 86.2 and 92.8 polymorphism percentage, respectively. SCoT markers provided more discriminating data. Cluster analysis based on collected data of protein, ISSR and SCoT succeeded in detection of genetic variety and heterogeneity within the genotypes. Therefore, Crosses between different genotypes based on Cluster analysis could lead to the development of effective breeding materials.

Keywords: Faba bean genotypes, evaluation, earliness traits, yield and its components, Protein Marker, ISSRs, SCoT Marker.

Introduction

Faba bean (*Vicia faba* L.) is an important legume crop produced worldwide; its high yield makes it attractive to the producers (Merga *et al*., 2019). In Egypt, faba bean seeds are considered an important food for humans and animals due to their high nutritional value. It is one of leguminous crops that are distinguished by its fixation of nitrogen, converting it into a suitable form for plants and increasing soil fertility, so it is planted alternately with the cereals (Mona *et al*., 2011). Faba bean seeds also have high export value for feed markets (Gong *et al*., 2011). The cultivated area with faba bean in Egypt was 131.428, 97.906, 89.707, and 81.934 thousand feddan with an average seed yield of 1.324, 1.423, 1.486, 1.469 and 1.449 ton/fed, in the seasons 2010/2011, 2011/2012, 2012/2013, 2013/2014 and 2014/2015, respectively. Meanwhile, the total area was 80 thousand fed in season of 2018. This indicates decreasing in planted area with faba bean. Therefore, it is necessary to find ways to increase the productivity of feddan. One of the most important ways is the selection programs for the new genotypes, which can be implemented in various research stations in Egypt. In this, the plant breeder uses crop yield and yield attribute traits (El-Hady *et al*., 1998).

El-Emam and Rabie (2015) evaluated ten faba bean genotypes in north Egypt and found that genotype 9 (Hybrid 10 x Giza 461) was the earliest in maturity dates and flowering. Meanwhile, genotype 1 (Giza 461 x Nubaria 1), 3 (Giza 716 x Giza 402), 5 (Nubaria 1 x Giza 2) and 8 (Hybrid 8 x Giza 461) surpassed in number of pods per plant and number of seeds per plant. As well, Qabil *et al*. (2018) evaluated five new and five old faba bean cultivars for yield and its attributes and earliness characteristics. They proved that there were significant variances between the tested genotypes for earliness characters.

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Ultimate aim of plant breeders is to produce cultivars with high yield potential, also consistent performance and extensive adaptability to environment. The genotype by environment interaction (G×E) is crucial for faba bean breeders. Strong G×E interactions for quantitative traits like seed yield can dramatically reduce the benefits of selecting superior genotypes (Fathi *et al.,* 2013). So, the assessment of genetic differences is a critical element of genetic studies, germplasm characterization, biodiversity research and choosing favorable genotypes in plant breeding programs. Measuring genetic variety at the DNA level by using molecular markers such as RFLP, AFLP, RAPD, , TRAP, and SSAP can be employed to evaluate genetic variations among V. faba genotypes (Abid *et al*., 2015; Akash *et al.,* 2017; Avramidou *et al.,* 2023a and Mahdy *et al.,* 2021). Molecular markers are reliable for selecting important agricultural characteristics, fast and unaffected by conditions of environment (Al-Hadeithi *et al*., 2021). Intersimple sequence repeat (ISSR) molecular marker technique was efficient in detected the genetic variety of V. faba genotypes (Wang *et al*., 2012; Terzopoulos and Bebeli, 2018; Asfaw *et al*., 2018).Alghamdi *et al.*, 2012a) indicated that ISSR is effective in estimating genetic variation and the relation between faba bean genotypes.

A rapid technique start codon targeted (SCoT) marker was advanced based on the short-conserved region flanking the start codon ATG in plant genes (Collard *et al*., 2009; Bhattacharyya *et al*., 2013). SCoT analysis was efficient technique for identify genetic variation and relationships between faba bean germplasm (Avramidou *et al*., 2023a). Bhattacharyya *et al.,* 2013; Guo *et al*., 2012 mentioned that a single primers were used as forward and reverse in SCoT markers, so it is similar to ISSR and RAPD Moreover, ISSR, RAPD and SCoT marker has low cost and is efficient to use.

The current study aimed to evaluate eighteen new faba bean genotypes for their earliness, yield and its components through mean performance as well as to determine the important traits linked to seed yield through correlation coefficient analysis. identifying genetic variation between the studied genotypes using biochemical (proteins) and molecular marker (ISSR and SCoT) techniques to test the efficiency of these techniques for genotype identification and marker-assisted selection, then help in management of germplasm and improvement current breeding methods to produce new cultivars in breeding programs.

Materials and Methods:

Field evaluation

The field study was conducted during the winter growing seasons 2020/2021 and 2021/2022 at the Department of Genetic Resources, Bahteem, Qalyubia Governorate, Egypt. The experiment aimed to evaluate eighteen faba bean genotypes for their growth, yield and yield attributes. Table 1 presented the pedigree and origin of the tested genotypes

A random complete block design with four replications was used in this experimental. Each plot contained 3 rows, 3 m long with a distance of 60 cm between rows. The planting was on single seeded hill and 20 cm a part. The cultural procedures for faba bean were applied as recommended.

The obtained data were recorded for 10 guarded plants from each genotype for the following characters: plant height (cm), number of branches/plant, days to first fertile nods, days to first flower, days to 50% flowering, days to end flowering, days to fruiting, number of fruiting nodes/plant, number of pods/node, number of pods/plant, pod length (cm), number of seeds /pod, number of seeds/plant, 100-seed weight (g) and seed yield/ plant (g).

The data were subjected to statistical analysis and simple correlation using Duncan's multiple range test (Duncan, 1955) at a 5% probability level. The mean values obtained were then compared using the Least Significant Differences (LSD).

Table 1. Pedigree and origin of the tested eighteen faba bean genotypes.

No.	Genotypes	Origin	Remarks
1	Giza2	Individual plant selection from local variety	Variety
2	114	Aswan- WadiKhrait	Landrase
3	136	Aswan- 19 km of Kalabsha	V. Good
4	168	Assiut- BeniFeez	Landrase
5	171	Qena	Landrase
6	174	Fayom- El Abbadla- Tamia	Low yield
7	175	Qena- 14 mm. East Nagahamady	Landrase
8	180 A	Dakhlia- 5 km. West MitCommr	White hilum
9	180 B	Individual plant selection from18 B	White hilum
10	180 C	Individual plant selection from18 B	Landrase
11	185	Gharbia- Tanta. 5km.est	V. Good
12	212	Fayom- Ezbat Khalil Ibrahim	Landrase
13	219	Fayom- 13 km. west El-Minia	Landrase
14	220	Beniswef	V. Good
15	223	El- MiniaBaniMazar 3 km	Landrase
16	Giza 716	461/842/83 x 503/ 453/83	Variety
17	Giza 3	Giza 1 x New Accession 29	Variety
18	T.W	Individual plant selection from Sudan	Landrase

Biochemical and molecular markers:

This part of research was performed in 2022 in the Laboratory of Genetics and Cytology Department, National Research Centre, Egypt.

Biochemical markers:

Protein analysis was performed by using 0.5 g seeds Samples of each genotype. Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE) was applied according to Laemmli, 1970. The 15% separating gel with protein Ladder

(BLUltraPrestained, GeneDirex) was used in electrophoresis apparatus (manufactured by Cleaver, UK). The obtained images were transmitted to the computer for analysis.

Molecular markers

DNA extraction

Germinate ten grains from each of the eighteen faba bean genotypes on wet filter papers for fifteen days , then extract genomic DNA from fresh leaves (0.5 g) using the DNAeasy® plant Mini kit (50) QIAGEN Germany.

Inter Simple Sequence Repeat (ISSR) and Start Codon Target (SCoT) analysis:

Seven ISSR and Six SCoT primers were used to achieve ISSR and SCoT fragments by PCR technique. (Table 2) lists the base sequences of these primers. PCR Master Mix (Go Taq G2 flexi DNA polymerase, Promega USA), was used to amplified a purified DNA, in Thermocycler (Bio-Rad, USA). Next, PCR products (12.5µl of each sample) were loaded into agarose gel (1.2%) stained with red safe staining, photographed and scanned using a Gel-Documentation system.

Table 2. List of seven ISSR and six SCoTprimers and their nucleotide sequences.

ISSR Primers		SCoT Primers	
Primer name	**Sequence(5'-3')**	**Primer name**	**Sequence (5'-3')**
ISSR-1	ACGAACACACACACACAC	**SCoT-12**	ACGACATGGCGACCAACG
ISSR-3	GGATGGATGGATGGAT	**SCoT-18**	ACCATGGCTACCACCGCC
ISSR-5	ACACACACACACACACC	**SCoT-19**	ACCATGGCTACCACCGGC
ISSR14	AGAGAGAGAGAGAGAGT	**SCoT-29**	CCATGGCTACCACCGGCC
ISSR-15	ACACACACACACACACCC	**SCoT-30**	CCATGGCTACCACCGGCG
ISSR-25	CACACACACACACACAAG	**SCoT-31**	CCATGGCTACCACCGCCT
ISSR-28	TCTCTCTCTCTCTCTCG		

Data analysis:

To determine the molecular weight and compare the presence and absence of each band in the protein, ISSR, and SCoT assay data, the total lab program was used. These data were then used in the Multi-Variant Statistical Package (MVSP) program (Kovach, 1998) to reveal the dendrogram and similarity matrix among the genotypes under study (UPGMA, using Nei&Li's coefficient, Nei and Li, 1979).

Results and Discussion

Earliness characters

Days to first fertile nods

Table 3 demonstrated that the differences between the tested genotypes were significant for days to first fertile nods in both seasons. Obtained results pointed out that the highest values of first fertile nods (4.80 & 4.88), (4.40 & 4.78), (4.00 & 4.65) were recorded with 175, 171 and 219 genotypes in 1st and 2nd seasons, respectively. While the lowest values of the first fertile nods (2.98 & 3.60), (2.78 & 3.85) were noticed with 174 and 180 B genotypes in the 1st and 2nd seasons, respectively.

Days to first flower:

Table 3 showed that there were significant differences between the tested genotypes for days to the first flower trait in the 1st and 2nd seasons. It is revealed that 82 G2 and 168 genotypes were the earliest ones, while 174, 180 A and 180 B genotypes were the latest ones in the two seasons.

Days to 50% flowering:

Data in Table3 cleared that the differences between genotypes were significant for days to 50% flowering. It is proved that 168 and 82 G2 genotypes were the earliest ones, while 174, 180 A and 180 B genotypes were the latest ones in the two seasons.

Days to end flowering:

The represented data in Table 3 for faba bean genotypes showed significant differences between the tested genotypes for days to end flowering in the 1st and 2nd seasons. However, the shortest periods for days to end flowering were noticed with 82 G2 and 212 genotypes, while the longest ones were resulted from 174, 180 A, 114 and 171 genotypes in the two seasons.

Days to fruiting:

The obtained results in Table 3 showed the days to fruiting trait as affected by the eighteen faba bean genotypes. The differences among genotypes were significant for this trait in both seasons. However,

the lowest values of days to fruiting (57.50 & 56.50 days), (58.50 & 59.50 days) were recorded in the 1st and 2nd seasons for 220 and 212 genotypes, respectively. Meanwhile, the longest days to fruiting were as a result of 180 A, 174 and 136 genotypes, respectively.

Table 3. Mean performance of the eighteen genotypes for earliness traits during 2020/ 2021 and 2021 / 2022 seasons.

Genotype	Days to first fertile nods		Days to first flower		Days to 50% flowering		Days to end flowering		Days to fruiting	
	1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season
82 G2	4.70	3.33	**39.75**	**31.25**	**39.75**	**39.25**	**44.50**	**45.00**	65.50	64.50
114	4.45	3.88	49.25	33.50	49.25	48.50	**55.25**	**55.75**	**70.00**	**69.00**
136	**3.98**	**3.58**	40.25	35.25	40.25	40.50	**55.00**	**55.50**	**70.00**	**71.00**
168	4.13	4.38	**39.00**	**34.75**	**39.00**	**38.75**	53.00	53.00	60.25	61.25
171	**4.40**	**4.78**	49.75	40.00	49.75	49.50	**55.00**	**56.00**	68.75	67.75
174	**2.98**	**3.60**	**55.00**	**48.50**	**55.00**	**55.25**	**64.00**	**66.00**	**71.50**	**70.50**
175	**4.80**	**4.88**	**50.75**	**42.75**	**50.75**	**51.50**	**55.50**	**56.00**	68.00	69.00
180 A	4.80	3.88	**54.75**	**46.25**	**54.75**	**54.75**	**60.25**	**60.75**	**71.25**	**72.25**
180 B	**2.78**	**3.85**	**50.00**	**43.25**	**50.00**	**50.00**	54.75	54.25	67.25	66.25
180 C	4.93	3.73	45.50	39.50	45.50	46.00	49.50	49.25	60.00	61.00
185	4.35	4.13	43.00	39.00	43.00	43.50	49.25	49.00	60.00	59.00
212	3.38	4.13	40.25	36.00	40.25	40.50	**45.00**	**45.25**	**58.50**	**59.50**
219	**4.00**	**4.65**	44.50	39.75	44.50	44.75	49.75	50.00	**70.75**	**69.75**
220	4.40	3.65	44.50	41.00	44.50	44.25	49.00	49.00	**57.50**	**56.50**
223	3.90	4.20	45.00	39.00	45.00	45.00	50.25	51.50	63.75	64.75
G 716	4.20	3.90	49.50	45.00	**49.50**	**49.00**	52.75	52.25	65.00	66.00
G. 3	3.73	3.90	49.25	42.00	49.25	49.00	52.75	51.50	65.25	64.25
T.W	3.55	3.20	48.75	35.00	48.75	49.25	**55.25**	**55.25**	68.50	69.50
L.S.D (0.05)	**0.67**	**0.69**	**1.98**	**1.43**	**1.98**	**2.02**	**2.14**	**1.86**	**1.953**	**1.953**

Yield and its attributes:

Plant height:

Table 4 showed significant differences among the eighteen tested genotypes for plant height trait in the 1st and 2nd seasons. However, the obtained results proved that the greatest values of plant height (132.50 & 122.50 cm), (112.50 & 123.75 cm), (102.50 & 128.75 cm) were recorded with 212, 174 and 171 genotypes in the 1st and 2nd seasons, respectively. Meanwhile, G 716 and 220 genotypes revealed the lowest values of plant height (88.85 & 81.25 cm), (105.00 & 85.00 cm) in the 1st and 2nd seasons, respectively.

Number of branches:

Table 4 showed differences among the eighteen genotypes were significant for number of branches in both seasons. The results revealed that the greatest values of number of branches (4.80 & 4.95), (4.73 & 4.75), (4.43 & 4.55), (4.50 & 4.45) were recorded with 180 B, 171, 212 and T.W. genotypes in the 1st and 2nd seasons, respectively. Meanwhile, the lowest values of the number of branches (2.80 & 2.75), (3.15 & 2.90) were noticed with 82G2 and 223 genotypes in the 1st and 2nd seasons, respectively.

Number of fruiting nods/plant:

Table 4 showed number of fruiting nods/plants affected by the tested faba bean genotypes. The differences among genotypes were significant for number of fruiting nods/plant in both seasons. The highest values of this trait (11.65, 12.15), (11.05 & 10.20), (10.95, 9.45) were recorded in the 1st and 2nd seasons for 212, 180 B and 223 genotypes, respectively. Meanwhile, the lowest values (4.23 & 3.35), (3.82, 4.38) were as a result of 136 and 168 genotypes, respectively.

Number of pods/ nod:

The presented data in Table 4 showed significant differences between the tested genotypes for number of pods/nod in both seasons. Obtained results pointed out that the greatest values of number of pods/nod (4.15 & 3.93), (3.50 & 3.63), (3.50 & 3.40), (3.25 & 3.25), (3.08 & 3.38) were recorded with G.3, 114, 220, 212, 180A and 180C genotypes in the 1st and 2nd seasons, respectively. While the lowest values of the pods/nod number (1.50 & 1.90), (1.78 & 1.90), (1.95 & 1.95) were noticed with 171, 168 and 82 G2 genotypes in the 1st and 2nd seasons, respectively.

Number of pods/ plant:

Table 4 showed that the differences between the studied genotypes were significant for the number of pods/ plant in the 1st and 2nd seasons. Results pointed out that the highest values of number of pods/ plant (28.50 & 27.25), (26.00 & 27.00), (26.50 & 25.50), (21.50 & 20.5), (20.50 & 19.50) were due to G3, 114, 220, 212 and 180 A genotypes in the 1st and 2nd seasons, respectively. Meanwhile, the lowest values of the number of pods/ plant (12.75 & 11.75), (12.50 & 12.50) were attained by 171 and 168 genotypes in the 1st and 2nd seasons, respectively.

Pod length:

Table 5 appeared the significant differences among the tested genotypes for pod length in the 1st and

2nd seasons. Results proved that the highest values of pods length (9.75 & 10.00), (9.75 & 9.50), (8.50 & 10.75), (9.00 & 8.75 cm), (8.75 & 9.00 cm) were due to G.3, 114, 220, 212 and 180 A genotypes in the 1st and 2nd seasons, respectively. Meanwhile, lowest values of the pod length (7.00 & 6.75), (7.25 & 6.75 cm) were attained by 171 and 168 genotypes in the 1st and 2nd seasons, respectively.

Number of seeds/pod:

Table 5 appeared the differences between the tested genotypes were significant for the number of seeds /pod in the 1st and 2nd seasons. Results appeared that the highest values of the number of seeds /pod (3.5 & 3.5) were due to G.3, 114, 220 and 212 genotypes in the 1st and 2nd seasons. Meanwhile, the lowest values of the number of seeds/pod (2.75 & 2.50), (3.25 & 2.50) were produced by 171 and 168 genotypes in the 1st and 2nd seasons, respectively.

Number of seeds/ plant:

Table 5showed the seeds number / plant as affected by the tested eighteen genotypes. The differences among genotypes were significant for number of seeds/ plant in the both of seasons. However, the highest values of this trait (57.50 & 56.50), (39.75 & 40.75), (39.00 & 39.50), (32.00 & 32.50), (31.50 & 30.50) were recorded in the 1st and 2ndseasons for G.3, 114, 220, 212 and 180 A genotypes, respectively. Meanwhile, the lowest values (12.50 & 11.75), (12.50 & 12.50) were recorded with 171 and 168 genotypes, respectively.

100 seed weight (g):

Table 5 demonstrated that the weight of 100- seeds was affected by the tested eighteen faba bean genotypes. The differences among genotypes were significant for the weight of 100 seeds in the two seasons. However, the highest values of this trait (107.15 & 108.63), (102.15 & 112.68), (106.73 & 106.85), (101.13 & 112.15), (109.58 & 101.30 g) were recorded in the 1st and 2nd seasons for G.3, 114, 220, 212 and 180 A genotypes, respectively. Meanwhile, the lowest values (76.10 & 71.13), (78.75 & 69.18 g) were recorded with 171 and 168 genotypes, respectively.

Table 4. Mean performance of the eighteen faba bean genotypes for yield and its attributes during the two seasons of 2020/ 2021and 2021 / 2022.

Genotypes	Plant height (cm)		Number of branches		Number of fruiting nods/plant		Number of pods/ nod		Number of pods / plant	
	1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season
82 G2	102.50	106.50	**2.80**	**2.75**	6.18	6.88	1.95	1.95	12.50	13.50
114	103.75	115.00	4.28	4.35	7.03	6.90	**3.50**	**3.63**	**26.00**	**27.00**
136	98.75	112.50	3.75	3.70	**4.23**	**3.35**	2.68	3.00	15.50	14.50
168	100.00	102.50	3.88	3.83	**3.82**	**4.38**	**1.78**	**1.90**	**12.50**	**12.50**
171	**102.50**	**128.75**	**4.73**	**4.75**	5.00	4.50	**1.50**	**1.90**	**12.75**	**11.75**
174	**112.50**	**123.75**	4.38	4.30	8.40	6.48	3.05	3.08	14.75	15.75
175	110.00	115.00	3.88	4.00	5.65	6.83	2.03	2.15	12.50	13.75
180 A	110.00	121.25	4.18	4.13	5.65	6.75	**3.25**	**3.25**	**20.50**	**19.50**
180 B	113.75	105.00	**4.80**	**4.95**	**11.05**	**10.20**	2.98	2.78	15.50	14.50
180 C	98.75	93.75	**3.13**	3.25	10.33	8.35	**3.08**	**3.38**	16.25	16.25
185	105.00	99.00	3.13	3.35	9.63	10.18	2.68	2.93	15.50	14.50
212	**132.50**	**122.5**	**4.43**	**4.55**	**11.65**	**12.15**	**3.35**	**3.50**	**21.50**	**20.50**
219	**111.25**	**116.25**	3.63	3.75	7.48	6.65	2.13	2.43	14.25	14.25
220	**105.00**	**85.00**	4.28	4.20	6.33	7.58	**3.50**	**3.40**	**26.50**	**25.50**
223	101.25	102.50	**3.15**	**2.90**	**10.95**	**9.45**	2.18	2.58	14.50	14.25
G 716	**88.75**	**81.25**	3.03	3.10	7.70	8.35	2.95	2.33	14.25	14.50
G. 3	95.00	120.00	3.75	3.80	10.50	8.48	**4.15**	**3.93**	**28.50**	**27.25**
T.W	93.75	95.00	**4.50**	**4.45**	8.70	9.03	2.20	2.00	14.00	14.25
L.S.D (0.05)	**3.66**	**3.77**	**0.80**	**0.77**	**1.14**	**0.96**	**0.56**	**0.52**	**1.47**	**1.45**

Seed weight/ plant (g):

Table 5 showed the seed weight/ plant was affected by faba bean genotypes under study. The differences among genotypes were significant for the seed weight/ plant in the two seasons. However, the highest values of this character (57.53 & 56.50), (46.75 & 45.50), (45.45 & 46.00), (44.95 & 43.45), (42.50 & 41.50 g) were recorded in the 1st and 2nd seasons for G. 3, 114, 220, 212 and 180 A genotypes, respectively. Meanwhile, the lowest values (14.35 & 13.35), (19.20 & 18.20 g) were recorded with 171 and 168 genotypes, respectively.

Correlation Analysis:

A valuable and informative technique is correlation coefficient analysis, which provides basic selection criteria, directs model dependence on yield and its components in field experiments, and illustrates the degree and depth of relationships between significant plant traits. However, information about the nature of the association is frequently lacking in the selection process for crop improvement, so the most crucial thing to understand is how different characters are associated (Desai *et al*., 1994).

Table 5. Mean performance of the eighteen faba bean genotypes for yield and its attributes during the two seasons of 2020/ 2021 and 2021 / 2022.

Genotypes	Pod length (cm)		Number of seeds /pod		Number of seeds/ plant		100-seed weight (g)		Seed weight/ plant (g)	
	1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season	1st season	2nd season
82 G2	7.00	7.25	3.00	3.00	18.75	17.75	89.58	77.25	20.68	19.93
114	**9.75**	**9.50**	**3.50**	**3.50**	**39.75**	**40.75**	**102.15**	**112.68**	**46.75**	**45.50**
136	8.75	8.50	3.00	3.25	25.75	26.75	104.43	103.40	33.85	32.85
168	**7.25**	**6.75**	3.25	2.50	**12.50**	**12.50**	**78.75**	**69.18**	**19.20**	**18.20**
171	**7.00**	**6.75**	2.75	2.50	**12.50**	**11.75**	**76.10**	**71.13**	**14.35**	**13.35**
174	8.75	8.75	3.25	3.00	29.50	27.25	100.90	107.43	36.18	35.18
175	6.50	8.75	3.00	3.00	21.50	20.50	92.73	84.35	**20.85**	**19.85**
180 A	**8.75**	**9.00**	**3.50**	3.00	**31.50**	**30.50**	**109.58**	**101.30**	**42.50**	**41.50**
180 B	9.00	8.50	2.75	3.50	28.25	27.25	105.30	102.80	34.50	33.50
180 C	9.00	8.75	2.75	3.50	30.25	31.25	104.85	103.60	39.18	37.75
185	8.50	8.75	3.50	2.75	25.00	26.00	102.38	102.25	32.78	31.50
212	**9.00**	**8.75**	**3.25**	**3.50**	**32.00**	**32.50**	**101.13**	**112.15**	**44.95**	**43.75**
219	8.25	8.25	3.00	3.00	20.75	21.75	101.85	94.40	25.50	24.50
220	**8.50**	**10.50**	**3.50**	**3.50**	**39.00**	**39.50**	**106.73**	**106.85**	**45.45**	**46.00**
223	8.75	8.25	3.00	3.00	22.00	21.00	100.00	100.35	27.25	26.00
G 716	8.50	8.75	3.00	3.00	21.75	22.75	100.33	103.48	29.38	28.25
G. 3	**9.75**	**10.00**	**3.50**	**3.50**	**57.50**	**56.50**	**107.15**	**108.63**	**57.53**	**56.50**
T.W	8.25	8.25	3.00	3.00	20.75	21.75	91.15	93.90	22.68	23.50
L.S.D (0.05)	**0.75**	**0.81**	**0.80**	**0.66**	**2.29**	**2.37**	**3.56**	**4.80**	**3.86**	**3.82**

Simple correlation coefficients between seven traits for eighteen faba bean (*Viciafaba* L.) genotypes in two seasons are demonstrated in Table 6. Correlation coefficients were significant ($P < 0.01$) between number of pods/ nod with each of number of seeds/pod (r = 0.77 and 0.76), number of seeds/ plant (r = 0.80and 0.75), 100 - seed weight (r = 0.76 and 0.80) and seed weight/plant (0.72 and 0.74) in the first and second seasons, respectively. Consequently, this observation suggested that selection practiced for the improvement number of pods/ nods would automatically improve these characters. Positive and significant correlation coefficients ($P<0.01$) between number of pods/plants and each of number of seeds/pod (r = 0.76 and 0.88), number of seeds/plant (0.79 and 0.78), 100-seedweight (r = 0.89 and 0.80) and seed weight/plant (g) (r= 0.78 and 0.74) in the first and second seasons, respectively.

***Genetic diversity based on biochemical marker*:**
The differences in total protein recorded by SDS-PAGE based on bands number among eighteen faba bean genotypes as shown in Figure (1). Variations were assessed depending on molecular weight (MW). A total number of 14 bands were obtained, ranging from (10-130kDa), three bands of which were polymorphic with 27.3 % polymorphism. The greatest number of bands (14 bands) appeared in four genotypes at same loci in their pattern (180A, 180B, G716 and) indicating highly similar genetic background, while 174 exhibited the lowest number of bands (11 bands).

Table 6. Correlation coefficients between studied yield characters of faba bean (*Viciafaba* L.) In the first (above diagonal) and the second seasons (bellow diagonal).

Characters	No. of pods/ nod			No. of pods / plant	Pod length	No. of seeds /pod	No. of seeds/ plant	100-seed weight	Seed weight/ plant
No. of pods/ nod		------		0.62	0.52	0.77*	0.80**	0.76**	0.72*
No. of pods/ plant		0.65		---------	0.57	0.76*	0.79**	0.89**	0.78**
Pod length		0.54		0.48	---------	0.44	0.76*	0.64	0.80**
No. of seeds /pod	0.76*	0.88**	0.32	---------	0.84**		0.87**		0.82**
No. of seeds/ plant	0.75*	0.78**	0.62	0.88**	--------		0.89**		0.91**
100-seed weight	0.80**	0.80**	0.63	0.80**	0.90**		----------		0.83**
Seed weight/ plant	0.74*	0.74*	0.85**	0.86**	0.94**		0.86**		-------

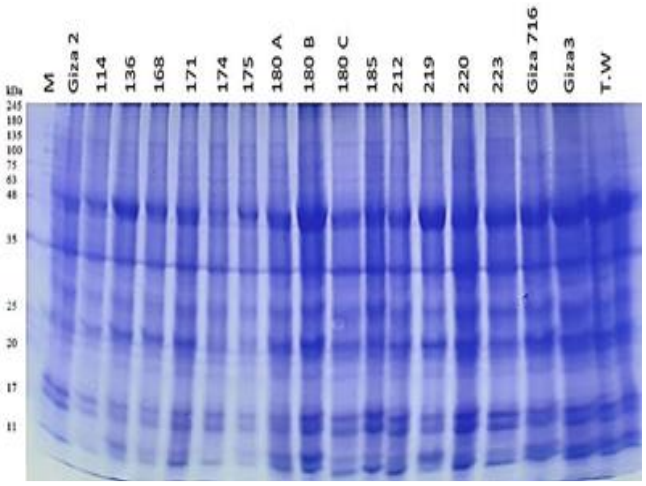

Fig. 1. Photograph of electrophoretic patterns of the eighteen bean genotypes for seedling total proteins performed by SDS-PAGE. M: is standard protein.

The ranged of genetic similarity was 0.88 to 1. Some genotypes manifested identical genetic similarity to others, such as 180C and each of 168, 136 and 171; 212 and each of 168, 136 and 171; while some genotypes such as 175 and 114 and also G2 41 and 175 revealed 88% similarity. The clusters analysis (Figure 2) categorized the genotypes into two clusters, only G2, 114 and 174 genotypes included in the first. While, two sub-clusters emerged from the second cluster: the first sub-cluster comprised genotypes 220, 217, 175 and the second sub-cluster had the remaining genotypes.

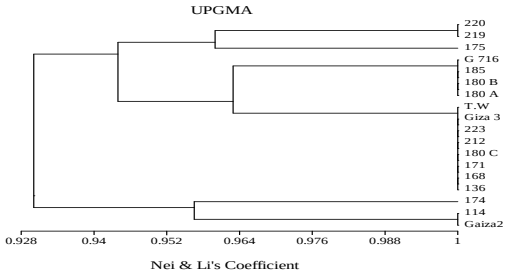

Figure 2. Dendrogram demonstrated the genetic relationship between the eighteen faba bean genotypes produced from seed storage proteins pattern.

Genetic diversity based on ISSR markers

Using seven ISSR primers with the eighteen genotypes revealed total number of bands 548 bands in 65 loci, 57 loci had a polymorphism of 89.3% (Table 7). The number of polymorphic bands generated per primer extends from 6 to 14 bands with sizes ranging from 122bp to 1600 bp. The genotype Giza 2 exhibited the highest number of fragments (40 PCR-amplified fragments) by using all primers, while genotype 114 gave the lowest number (22 fragments), as shown in Figure (3) and Different numbers of amplified fragments were scored in the other genotype where primer ISSR-14 presented the highest number for all studied genotype (116 fragments), while primer ISSR-15 showed the lowest number (43 fragments).

Specific band of MW 1190bp was detected in the electrophoretic pattern of genotype G2 using primer ISSR-1 (Figure 3). In addition, another specific band at MW 1250 bp was detected in the pattern of genotype 174 and 920 bp in line 180A using primer ISSR-3. One specific band at MW 1000bp using primer ISSR-5 was presented in genotype Giza 3.

Two specific bands at MW 1600bp and 1350 bp appeared in genotype 185 using primer ISSR-14. ISSR-15 primer manifested one specific band at MW 1400bp in the patterns of genotype 180C. In another hand, ISSR-28 primer revealed the highest number of specific marker (5 bands) two of them detected in genotype 219 at MW 300 and 1500bp, two fragments appeared in genotype 220 at MW 500 and 1000 bp, another one detected in genotype 174 at MW 850bp. Table 7 presented the polymorphism detected by the seven ISSR primers used to identify the tested genotypes. ISSR-14 Primer gave the highest number of loci in all genotypes (15 fragments), while primer ISSR-15 showed the lowest number (6 fragments).

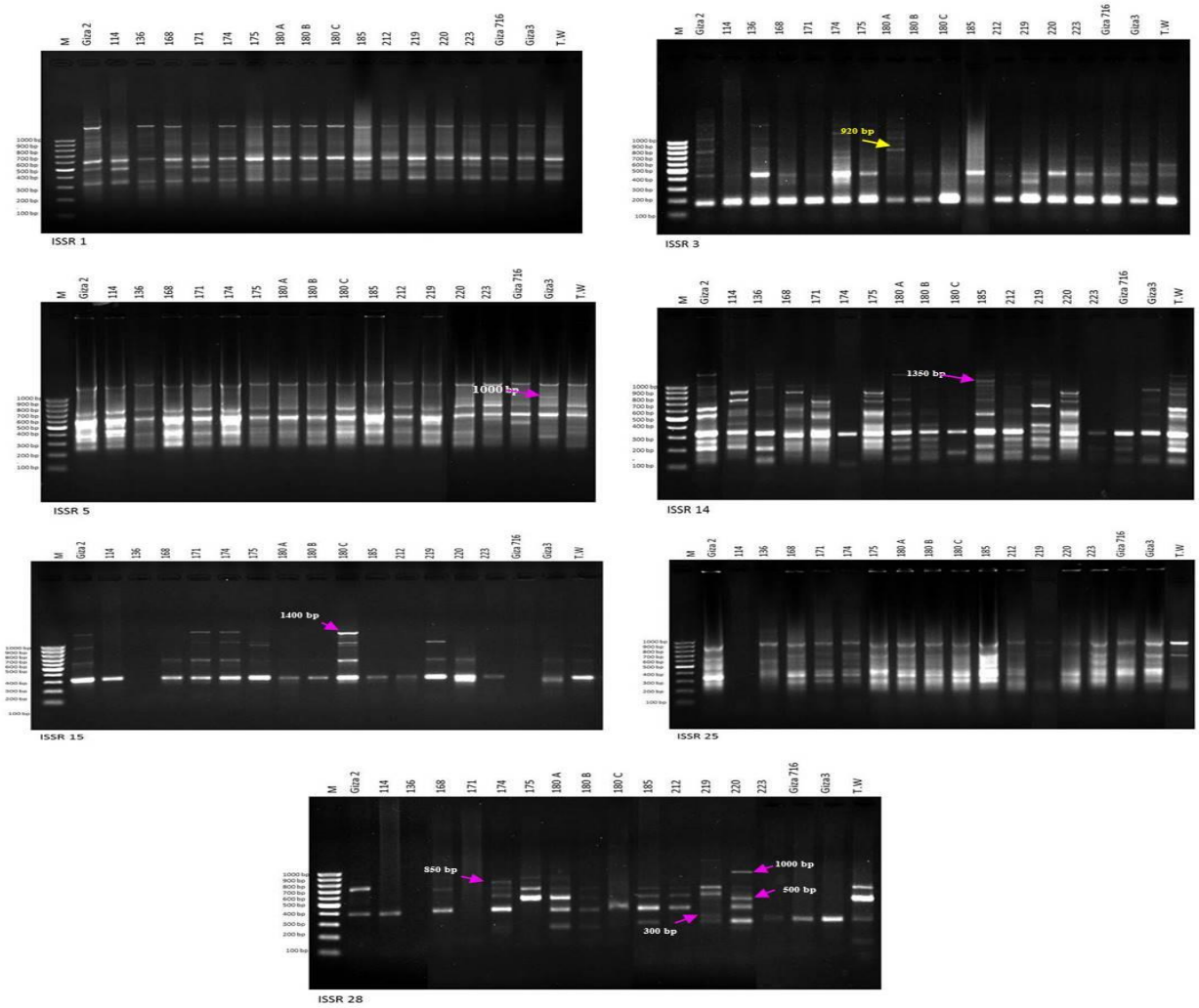

Fig. 3. Electrophoretic profiles of PCR-DNA products using seven ISSR primers with eighteen faba bean genotypes M: DNA ladder markers ranged in size from 1000-100 bp.

The ISSR markers of the primer ISSR-14 and ISSR- 28 succeeded in characterizing eighteen faba bean genotypes into eighteen different patterns, in which each genotype appeared in a unique pattern. In addition, genotypes 136 did not manifest any band using primer ISSR- 28. Also genotype L114 did not manifest any band using in Primer ISSR-28. Therefore, these findings demonstrated that the genotypes under study have various genetic backgrounds, so this analysis effective in analyzing genetic diversity in genotypes

The genetic similarity of the studied genotypes resulting from ISSR analysis (extended from 0.49 to 0.88), where 180B and 180A gave the highest genetic similarity (88%), while genotypes 136 and 114 displayed the lowest genetic similarity 0.49. dendrogram (Figure **4)** showed that the genotypes branched into two clusters. only one genotype

Table 7. The polymorphic loci and specific band values in eighteen faba bean genotypes as revealed by ISSR markers.

Primer	loci	range sizes of loci	Polymorphic bands	Monomorphic bands	Polymorphism (%)	No. of specific bands	Total bands in all genotypes
ISSR-1	8	298-1500	6	2	75	1	85
ISSR-3	9	200-1500	8	1	88.8	1	64
ISSR-5	10	245-1553	7	3	70	1	115
ISSR-14	15	122-1600	14	1	93.3	2	116
ISSR-15	6	300-1510	5	1	83.3	1	43
ISSR-25	7	290-900	6	1	85.7	0	80
ISSR-28	10	321-1498	10	0	100	5	47
Total	65	122-1600	56	9	86.2	11	548

(136) was included in The first, while second can be separated into two sub-clusters; only one genotype (114) was included in first sub-clusters; the second classified into two group; (G3, G716 and 223) genotypes were included in the first group, on the other side the second group contained the remaining of genotypes

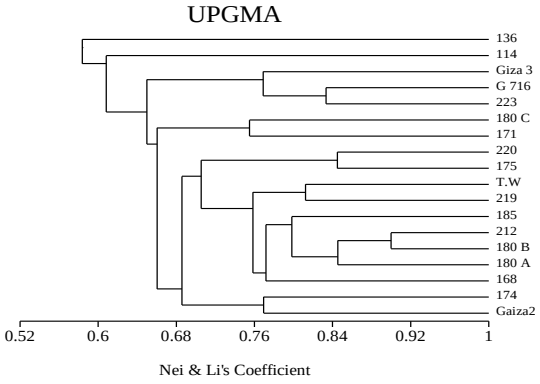

Fig. 4. Dendrogram demonstrated the genetic relationship between the eighteen faba bean genotypes based on ISSRs analysis.

Genetic diversity based on SCoT markers

All SCoT primers exhibited distinct and reliable band patterns (Figure 5). The whole number of bands resulted from using six SCoT primers with the eighteen genotypes was 826 bands at 84 loci, of which 76 loci were polymorphic with a polymorphism of 92.8 % (Table 8). The number of Polymorphic bands produced per primer extended from 9 to 15 bands with ranged size from 100 to 4500 bp. The genotype 175 presented the greatest number of fragments (56) by using SCoT primers, however genotype 171 gave the lowest number (23 fragments).The highest number of amplified fragments (162bands produced by SCoT-29 Primer for all studied genotypes under study, while the lowest number of fragments (119 fragments) revealed by SCoT-12 primer.

Six specific markers with different molecular weights were presented in four genotypes; SCoT-12 (3000 and 163 bp), SCoT-29 (1750 bp) in genotype 185, SCoT-18 (1122 bp) in genotype 175 and 245 bp in genotype 180C, SCoT-19 (700bp) in genotype Giza 3and SCoT-31 (2600bp) in genotype 220. SCoT analysis using primer SCoT-12 illustrated one specific ISSR marker of 1000bp in the patterns of genotype 185 and 219. The six SCoT primers revealed polymorphism used for the identification of studied genotypes. Primer SCoT-18, SCoT-29 and SCoT-30 gave the highest polymorphic loci in all genotypes with 100 % polymorphism, while primer SCoT-12 showed 85.7% polymorphism.

Most of SCoT Primer succeeded in characterizing eighteen genotypes into eighteen different patterns, in which each genotype appeared in a unique pattern, while the genotypes Giza 2 and 171 did not manifest any band with SCoT-30 also the genotype Giza 2 did not manifest any band with SCoT-29. Therefore, these results indicated that the genotypes under study have various genetic backgrounds, and the most of primer used in SCoT-PCR analysis succeeded in distinguishing the eighteen faba bean genotypes in a unique banding pattern for each variety, and this analysis is effective in analyzing genetic diversity in the studied genotypes.

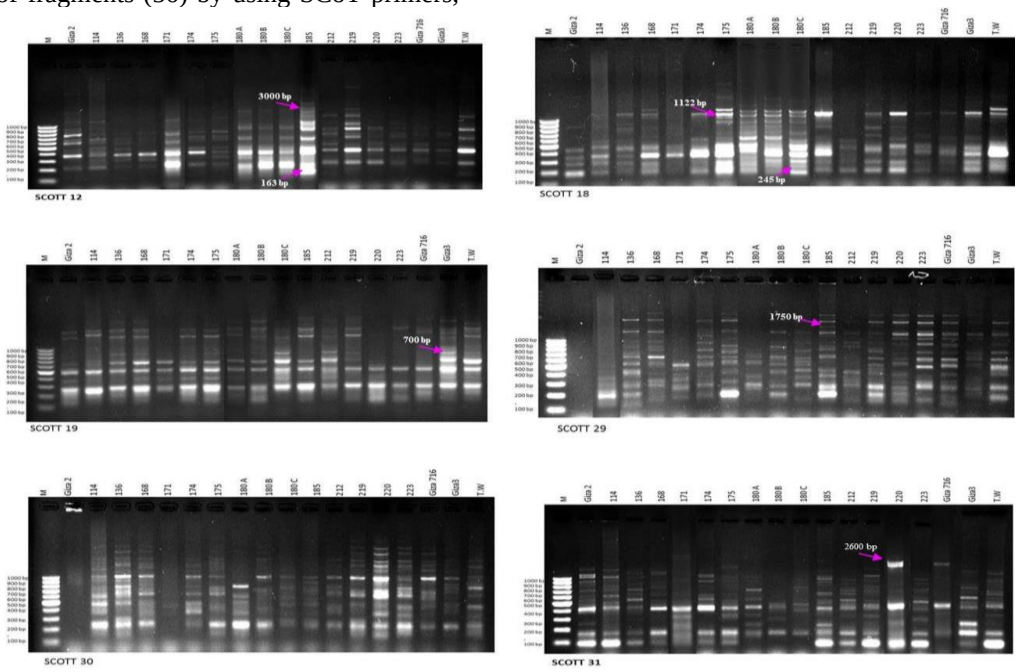

Fig. 5. Electrophoretic profiles of PCR-DNA products by using six primers of SCoT with eighteen faba bean genotypes M: DNA ladder markers ranged in size from 1000-100 bp.

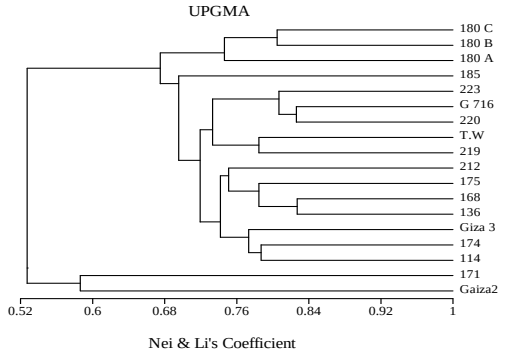

Fig. 6. Dendrogram demonstrated the genetic relationship between the eighteen faba bean genotypes based on SCoT analysis.

Dendrogram tree of the studied genotypes based on SSRs analysis was shown in **Figure (6) where** the genetic similarity extended from 0.36 to 0.83. Genotypes G716 and 220 gave the highest genetic similarity 0.83. On other hand, G716 and G2 displayed the lowest genetic similarity (0.36). The eighteen genotypes were separated into two clusters, the first had the genotypes (171 and Giza2), while the second was branched into two sub-clusters; three genotypes (180C, 180B and180A) was presented in the first (sub-cluster). The remaining genotypes were presented in the second (sub-cluster)

Combined results of biochemical and molecular markers:

The dendrogram and genetic similarity index based on combined results of protein, SCoT and ISSRs analysis for the eighteen faba bean genotypes were displayed in Figure (7) and Table (9). The values of similarity exhibited substantial differences ranging from 53 to 85 %. Some characteristic genotypes gave great values of genetic similarity with others, for example, 223 and G 716 (85%), 180A and 180 (82%), and 219 and T.W (82%). However, some varieties showed slightly low genetic similarity, such as G2 and G716 (53%), G171 and G136 (58%), and G171 and G716 (58%). The dendrogram separated the eighteen genotypes into two clusters; one of them has only three genotypes (G171, 114 and G2). Another one was branched into two sub clusters; the first included two genotypes (G175 and 220), while the second branched into two groups. The four genotypes G3, G716, 223 and 174 were presented in first group, and the second group was branched into two subgroups. three genotypes 180C, 180 B and 180 A with a similarity of 85% were present in first (sub-group), while the other one contained the rest six genotypes T.W, 219, 185, 212,168 and G136 with a similarity of 82% for T.W and 219, with 88% for 212 and 168. The results obtained confirmed that both of biochemical and molecular markers (ISSR and SCoT) succeeded in identifying the genotypes and detecting the genetic relationships between them.

Table 8. The polymorphic markers loci and specific band values in eighteen faba bean genotypes as revealed by SCoT marker.

Primer	Loci	Range sizes of loci	Polymorphic bands	Monomorphic bands	Polymorphism (%)	No. of specific bands	Total bands in all genotypes
SCot-12	14	163-4500	12	2	85.7 %	2	119
SCoT-18	15	220-4100	15	0	100 %	2	131
SCoT-19	11	195-2600	9	2	81.8 %	1	125
SCoT-29	15	200-2645	15	0	100 %	1	162
SCoT-30	14	253-4100	14	0	100 %	0	137
SCoT-31	15	100-2590	13	2	86.7 %	1	152
Total	84	100-4500	78	6	92.8 %	7	826

Table 9. Percentages of genetic similarity for the eighteen faba bean genotypes based on protein, ISSR and SCoT banding patterns.

	Gaiza2	114	136	168	171	174	175	180 A	180 B	180 C	185	212	219	220	223	G 716	Giza 3	T.W
Gaiza2	1.00																	
114	0.70	1.00																
136	0.65	0.74	1.00															
168	0.67	0.74	0.79	1.00														
171	0.70	0.73	0.58	0.66	1.00													
174	0.73	0.71	0.75	0.76	0.65	1.00												
175	0.59	0.70	0.72	0.76	0.62	0.69	1.00											
180 A	0.71	0.69	0.72	0.73	0.69	0.72	0.74	1.00										
180 B	0.62	0.67	0.73	0.78	0.62	0.70	0.78	0.82	1.00									
180 C	0.63	0.67	0.69	0.72	0.67	0.70	0.73	0.77	0.81	1.00								
185	0.70	0.71	0.73	0.78	0.66	0.74	0.71	0.77	0.76	0.70	1.00							
212	0.65	0.74	0.76	0.80	0.67	0.74	0.73	0.76	0.80	0.72	0.79	1.00						
219	0.69	0.73	0.73	0.75	0.61	0.74	0.75	0.72	0.79	0.70	0.77	0.79	1.00					
220	0.62	0.74	0.70	0.72	0.65	0.70	0.77	0.70	0.74	0.69	0.67	0.70	0.74	1.00				
223	0.62	0.72	0.73	0.76	0.65	0.78	0.71	0.69	0.74	0.71	0.74	0.77	0.72	0.76	1.00			
G 716	0.53	0.68	0.74	0.75	0.58	0.71	0.68	0.65	0.74	0.68	0.69	0.73	0.72	0.76	0.85	1.00		
Giza 3	0.63	0.73	0.76	0.76	0.61	0.76	0.73	0.74	0.75	0.73	0.74	0.77	0.72	0.74	0.80	0.76	1.00	
T.W	0.66	0.72	0.75	0.78	0.65	0.70	0.75	0.74	0.81	0.68	0.75	0.76	0.82	0.76	0.74	0.71	0.76	1.00

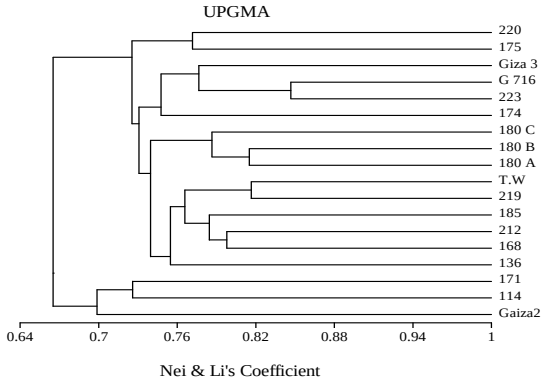

Fig. 7. Dendrogram demonstrated the genetic relationship between the eighteen faba bean genotypes baed on combined protein, ISSR and SCoT analysis.

Table (10) regarding the combined data of proteins, ISSRs and SCoT analysis**,** SCoT analysis achieves that the highest polymorphism percentage was 92.8% and seven specific fragments, while, proteins fingerprint gave the lowest polymorphism percentage (27.3 %) with no unique fragment. Meanwhile, ISSRs analysis revealed 86.2% polymorphism and produced the highest number of specific bands (11 fragments).

Table 10. Comparison between efficiency of the studied protein, ISSRs and SCoT markers for distinguishing among eighteen faba bean genotypes.

Molecular Parameter	Value		
	Protein	ISSR	SCoT
Total Bands	14	65	84
Monomorphic Bands	11	56	6
Polymorphic Bands	3	9	78
% of Polymorphism	27.3	86.2	92.8
Unique Bands	-	11	7

Discussion

Several previous studies showed that there are high significance differences between the faba bean genotypes for the plant height, number of branches/plant, pod-length, number of pods/plant, number of seeds/pod, number of seeds/plant, 100-seed weight and seed yield/plant (Ahmed *et al*., 2013; Sharifi, 2014; Mitiku and Wolde, 2015; Tamene *et al*., 2015; Verma *et al*., 2015; Degife and Kiya, 2016; Hamza, 2017; Hamza and Khalifa, 2017 and Qabil *et al*., 2018)

Also, it demonstrated positive and significant correlation ($P < 0.01$) between pod-length (cm) with seed-weight/plant (g) (r = 0.80 and 0.85), number of seeds/pod with seed weight/plant (r = 0.82 and 0.86), number of seeds/plant with seed-weight/plant (r = 0.91and 0.94) and 100-seed weight with seed weight/plant (r = 0.83 and 0.86) in the two years (Ahmed *et al*., 2013; Verma *et al*., 2015; Hamza, 2017; Kumar *et al*., 2017; Qabil *et al*., 2018). Mitiku and Wolde (2015) proved that number of seeds/pod with seed yield/ plant was positively and significantly correlated. Meanwhile, the results showed positive and insignificant between number of pods / plant with number of pods/ nod and pod length (r=0.62 and 0.65) and (r=0.52, 0.54) in two seasons, respectively. Also, 100-seedweight trait with pod length (r=0.64and 0.62). These results are in consistent with those reached by El-Kady and Khalil (1979), Ebrahim *et*

al. (2012) and Bakhiet *et al.* (2015).Assessing genetic variation in crop populations using morphological traits only is not an easy task because morphological traits can be influenced by environmental factors and cultivation conditions. Certainly, protein analysis (SDS-PAGE) (Hou *et al*. 2015) and molecular markers are increasingly being used to detect the differences in DNA level between and within crop populations (Hoxha *et al.* 2004). Characterization of DNA is effective approaches in the genetic improvement(Brake *et al.* 2021). To estimate genetic variation between genotypes in plant breeding programs, molecular markers must be used (Porthand ElKassaby 2014), identifying quantitative trait locus (Li *et al*. 2014) and marker-assisted selection (Sakiyama *et al*. 2014).

Morphological traits and different types of markers were applied in this work; total protein as a biochemical marker and two types of molecular markers (ISSRs and SCoT) to characterize a set of the genetic diversity of the studied genotypes. We observed that SCoT gave a high polymorphism percentage compared with protein and ISSR marker, which were in an consistent with those of Albrifcany et al 2022 who assess molecular differences among 13 genotypes of Vicia faba from Kurdistan and Egypt for use in crop breeding programs in Kurdistan and found that SCoT gave a high polymorphism percentage compared with ISSR marker. Avramidou et al., 2023bfound that SCoT markers are appropriate tool for identify genetic diversity between fifty three Greek, varied faba bean populations.

Several studies have indicated that SCoT marker is gene targeted, so it considered more effectively than other dominant DNA molecular markers such as RAPD and ISSR(Gupta *et al*., 2018). Also, SCoT can generate both dominant and co-dominant markers resulting from sequence variations, insertions and deletions, respectively (Salazar Laureles *et al.,* 2015 and Aswathy *et al*., 2017). SCoT markers have many advantages: it has simple process does not expensive, has high polymorphism, and suitable for molecular-assisted breeding. Nevertheless, Using SCoT and ISSR markers together provides, reliable and efficient results in studying genetic diversity than using individual marker(Mao *et al*., 2018). The collective data of molecular marker (SCoT and ISSR) were useful for estimating the genetic diversity and relationships between genotypes (Abd ELAziz *et al.,* 2019 and Osman and Ramadan 2020)

The similarity matrix of the combined data for protein, ISSRs, and SCoT marker is different from that of each marker individually; this indicated that each marker has a unique characteristic in distinguishing genotypes based on of genomic DNA amplification site. Therefore, molecular and biochemical markers can be performed either separately or together to study genetic variations in faba bean.

6. Conclusion

Molecular breeding is considered one of the branches of modern breeding, so field data was combined with molecular genetics in one work to characterize Faba bean breeding on a molecular basis, as it found differences between genotypes based on molecular markers ISSR and SCOTs with field data to achieve discipline in Faba bean breeding.

Consent for publication:

All authors declare their consent for publication.

Author contribution:

The manuscript was edited and revised by all authors.

Conflicts of Interest:

The author declares no conflict of interest.

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