III.5. Molecular and genetical studies of some promising faba bean lines under soil salinity stress at Nubaria region.

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ABSTRACT

The current investigation was carried out at Nubaria Agricultural Research Station, Agricultural Research Center, Egypt, during 2015/16 and 2016/17 seasons to evaluate 20 faba bean (Vicia faba L.) promising lines and five cultivars (Misr 3, Nubaria 1, Giza 843, Sakha 3 and Giza 716) under soil salinity stress. The entries were sown in a randomized complete block design (RCBD) with five replicates; the EC (dS m^{-1}) of the soil was 6.67 in the first season and 4.35 in the second one. The promising line M6 produced the greatest numbers of branches (4.13 and 4.80), pods (10.47 and 36.80), seeds (29.20 and 102.00) and seed yield $plant^{-1}$ (18.58 and 70.82 g) in the two seasons, respectively. Meanwhile, , Giza 843 cv. Recorded almost similar pod number (10.60 and 22.20), seed number (24.5 and 70.8) and seed yield $plant^{-1}$(17.56 and 64.94 g.), in the two seasons, respectively. Therefore, promising line M6 should be further evaluated in the advanced yield trials to release it as a new cultivar, to be grown under soil salinity stress. The correlation coefficient confirmed the highly significant positive correlation (0.98^{**}) between both of No. of pods $plant^{-1}$ and seed yield $plant^{-1}$ with No. of seeds $plant^{-1}$. The estimated value of heritability in broad sense was high for all characteristics in both seasons except for No. of branches $plant^{-1}$ in the first season, which was low (30.0). High heritability indicated that selection based on means would be successful in improving these traits. Expected genetic gains from selecting the top 5% of the characteristics under study as a percent of the mean was high except for plant height in the second season (18.5 cm) and No. of branches $plant^{-1}$ in the first season (9.2). High heritability estimates along with high genetic advance for seed yield and its attributes indicated an additive gene action in its inheritance. Total protein fractionation using SDS-PAGE was used. SDS-PAGE analysis of the investigated faba bean protein has demonstrated a great heterogeneity in the separated protein fractions. Proteins have specific bands referred to different saline conditions and differences within genotypes. Three RAPD primers were successful in generating reproducible and reliable amplicons for the twenty five faba bean genotypes.

Keywords: Vicia faba, Correlation Coefficient, Heritability, Genetic gain, SDS-PAGE analysis, RAPD markers.

INTRODUCTION

Faba bean, broad bean or field bean (*Vicia faba* L.; 2n = 12) is a major food and feed grain legume owing to the high nutritional value of its seeds, which are rich in protein 27-34% (***Duc, 1997***). It is considered as one of the major sources of cheap protein and energy in Africa, parts of Asia and Latin America, where most people cannot afford meat sources of protein (***Alghamdi, 2009*****).** In Egypt, faba bean is among the main nutritional sources of plant proteins (***Bakry* et al., *2011***). It ranks as the fourth most important legume crop in the world after dry beans, dry peas and chickpea (***Toker, 2004***). A rich and diverse germplasm collection is the backbone of successful crop improvement for increased crop production. Genetic resources have played a major role in providing source of resistance to biotic and abiotic stresses. It is important, not only to collect genetic resources, but also to evaluate, document and utilize these materials for their immediate and long-term use in breeding programs.

The average cultivated area over the last five years (2012-2017) was about 113,000 feddans with an average yield of 9.2 ard. fed.$^{-1}$, the total production was 119,000 t and the consumption was 420,000 t, so the total production of this crop is still limited and fails to cover the increasing local consumption. There is a prerequisite to enlarge the production by expansion throughout reclaimed areas, which signify the scope of cultivated lands (***Khalafallah* et al.*, 2008 and Bakry* et al., *2011***).

Nile River is the main source of fresh water in Egypt and is limited to 55.5 billion cubic meters annually, evidencing the need for alternative sources of water if irrigation needs are expanded. Agricultural drainage water or treated waste water is non-conventional water sources for irrigation but restrictions due to salinity-related issues limit their reuse for irrigation. Worldwide, the problem of salinization is steadily increasing and is more common in arid and semi-arid regions (***Evangelou and McDonald, 1999***). By the end of the 20th century about a third of the irrigated land was already affected by salinity (***Jacoby, 1999***), because of the use of poor quality water for irrigation and poor drainage. Globally, salinity has reached 19.5% and 2.1% in irrigated and dry land areas, respectively (***FAO, 2000***).

Salinity damages soil structure and decreases the productivity of most crops as plant growth is affected in several aspects of its metabolism. Salt tolerance of plants is of great economic and scientific importance. The economic impetus for research and development derives from the fact that salt-effected soils occupy about 10% of the world´s arable land (***Tanji, 1990***). ***Rush and Epstein (1976***) have argued that crop production could be greatly enhanced by selecting salt resistant strains. Difference in salt tolerance among species has been long recognized (***Mass and Hoffman, 1977***). In legumes, salt stress is significantly limits productivity (***Delgado* et al., *1994***). In general, legumes are either sensitive or moderately tolerant to salinity (***Mass and Hoffman, 1977***). Broad bean (*Vicia faba* L.) is moderately tolerant (***Maas and Hoffman, 1977, El-Karauri, 1979 and Katerji* et al., *2003***). Faba bean plants are more sensitive to water stress than other grain leguminous species (***Amede and Schubert, 2003 and Khan* et al., *2010***). It is more sensitive to salinity during early vegetative stages (***Al-Tahir and Abdulsalam, 1997***) and reduction in growth can be as much as 50 percent at 6.7 dS m^{-1} (***Maas and Hoffman, 1977***).

Pulses are considered to be more sensitive to salinity than cereals (***Maas and Hoffman 1997, Ayers and Westcot, 1985, Francois and Maas, 1994, Maas and Grattan, 1999 and Katerji* et al., *2001***). The reason for this higher sensitivity can be explained by several factors, the most important, the leguminous species have an undetermined growth cycle and, consequently, a reproductive stage that is longer than that of cereals (***Katerji* et al., *2005a, b***).

In Egypt, the faba bean is very important as reclamation crop for new land. This land is suffering from salinity as a main problem. The local cultivars vary from moderately sensitive to moderately tolerant to salinity. The productivity of these cultivars is severe decreasing under markedly salinity (***Soliman* et al., *2005***).

Mutation breeding using physical and chemical mutagens is considered to be one of the useful tools for plant improvement. Radiation mediated *in vitro* mutagenesis and selection has been successfully used to improve physiological traits such as salinity and drought tolerance in different crop plants (***Azzam and El-Sawy, 2005, Soliman* et al., *2005, Azzam* et al., *2007, Azzam and Khalifa, 2016 and Abdalla* et al., *2017***).

Recently, DNA-marker approaches have become gradually more utilized for taxonomic and phylogenetic analyses. They are not affected by environmental factors or by plant developmental stages. Besides, these approaches have potential for the routine testing of the genetic diversity and purity of accessions held in germplasm collections (***Gilbert* et al., *1999).*** Molecular markers, based on the polymerase chain reaction (PCR) technique, are the most commonly used for these purposes, several PCR –based techniques have been developed during the last two decades, each with specific advantages and disadvantages. Inter-simple sequence repeat (ISSR) markers permit detection of polymorphisms in inter- microsatellite loci, using a primer designed from dinucleotide or trinucleotide simple sequence repeats. ISSR analysis has been successfully documented to determine genetic diversity and relationships in faba bean (***Afiah* et al., *2007; Terzopoulosa and Bebeli, 2008; El-Sayed* et al., *2013; Afiah* et al., *2016; and Abdel-Salam, 2017***). The use of molecular tools has made possible the identification of differences on tolerance to salt within a crop. For instance, ***Afiah* et al. *(2007***) used Random Amplified Polymorphic DNA (RAPD) for genotypic identification of broad bean tolerant to drought**. *Waly* et al. *(2012***) used RAPD markers to detect the genetic variability and relationships among five faba bean lines**. *Khan* et al. *(2013***) used RAPD technology to investigate the influence of genetics on salt tolerance in 10 soybean genotypes concluding that variations on tolerance to salt can be partially accounted by plant physiological measures. ***Abdelraouf* et al. *(2016***) used RAPD to classify five faba bean cultivars into tolerant, moderately tolerant and sensitive. More recently**, *Abdel-Salam (2017***) used combination of RAPD and ISSR data to estimate the genetic similarity values among four faba bean cultivars and 14 newly developed tolerant genotypes up to 100 mM. Sodium chloride solution

Therefore, the objectives of this study, were to: (i) evaluate the performance of 25 genotypes of faba bean under salinity stress, (ii) estimate some genetic parameters such as heritability, genetic advance, GCA, PCV and correlation coefficient, and (iii) measure genetic distance and generate molecular profile for important faba bean genotypes using SDS-PAGE and RAPD markers.

MATERIALS AND METHODS

Experimental procedures

The present study was carried out at Nubaria Agricultural Research Station, ARC, Egypt during two successive winter seasons, 2015/16 and 2016/17. Twenty five faba bean (*Vicia faba* L.) genotypes were used that comprised five local varieties and twenty promising lines. The pedigree and some important characteristics of these genotypes are given in Table (1). The seeds were sown in a randomized complete block design (RCBD) with 5 replicates. Seeds were planted in single seeded hills, 20-cm apart. Each genotype was presented by one row, 3-meter long and 60-cm. in between. Some physical, chemical analysis and soil fertility parameters of the soil in the two seasons 2015/16 and 2016/17 are shown in Table (2).

Table 1. The pedigree of the genotypes used in the study.

No.	Genotype	Pedigree
1	**Misr 3 (P1)**	L.667 X (C. 241 X G. 461).
2	**Nubaria 1 (P2)**	Individual selected plant from Spanish variety Reina blanca.
3	**Giza 843 (P3)**	(561/2076/85 SKH X 461/485/83).
4	**Sakha 3 (P4)**	Individual selected plant from Giza 716
5	**Giza 716 (P5)**	Crossing between (416/842/83 X 503/453/83
6	**H1**	Misr 1 X (x-1714)
7	**H2**	Misr 1 X Misr 3
8	**H3**	Misr 1 X Nubaria 1
9	**H4**	Misr 1 X Giza 40
10	**H5**	(x-1714) X Misr 3
11	**H6**	(x-1714) X Nubaria 1
12	**H7**	(x-1714) X Giza 40
13	**H8**	Misr 3 X Nubaria 1
14	**H9**	Misr 3 X Giza 40
15	**H10**	Nubaria 1 X Giza 40
16	**M1**	Giza 714 treated with 30 Gy of gamma rays
17	**M2**	Giza 714 treated with 30 Gy of gamma rays
18	**M3**	Improved G3 treated with 60 Gy of gamma rays
19	**M4**	Improved G3 treated with 0.001 M concentration of sodium azide
20	**M5**	Improved G3 treated with 90 Gy of gamma rays
21	**M6**	Giza 716 treated with 120 Gy of gamma rays
22	**M7**	Improved G3 treated with 30 Gy of gamma rays
23	**M8**	Giza 716 treated with 0.001 M concentration of sodium azide
24	**M9**	Giza 716 treated with 30 Gy of gamma rays
25	**M10**	Giza 716 treated with 60 Gy of gamma rays

Table 2. Some soil physical, chemical analysis and soil fertility at the two seasons 2015/16 and 2016/17.

Season	EC dS m^{-1}.	PH	Anionic and cationic composition (meq/100g soil)				Soil fertility A.V. (ppm)		
			Anions						
			Ca^{+2}	Mg^{+2}	Na^{+}	K^{+}	N	P	K
2015/2016	**6.67**	8.31	24.15	2.13	38.71	3.71	30.62	3.7	83
2016/2017	**4.35**	8.31	14.74	2.64	24.13	1.94	37.35	3.71	87.2
Season	**Total $CaCo_3$%**	**Texture grade**	**Cations**				**Mechanical analysis**		
			Cl^{-}	HCO_3^{-}	CO_3^{--}	SO_4^{2-}	**Sand %**	**Silt%**	**Clay%**
2015/2016	**23.4**	Sandy clay loam	40.23	10.06	-	18.42	52.43	26.22	21.35
2016/2017	**24.17**	Sandy loam	27.12	6.21	-	10.17	56.13	28.95	14.92

The recommended cultural practices for faba bean production were adopted. Plant height (cm), number of branches $plant^{-1}$, number of pods $plant^{-1}$, number of seeds $plant^{-1}$, seed yield $plant^{-1}$ (g) and 100-seed weight (g) were estimated.

Estimation of genetic parameters

The mean values of the recorded data were subjected to analysis of variance according to ***Gomez and Gomez (1984***). The mean squares were used to estimate genotypic and phenotypic variance according to ***Sharma (1998).*** Differences among means were identified using Fisher's Least Significant Difference (LSD) test at the 0.05 probability level. Phenotypic and genotypic coefficients of variation, heritability in broad sense (H^2) and Genetic advance (GA%) for each characteristic were estimated by using variance components method (***Fehr, 1987***).

The genotypic and phenotypic coefficients of variation were estimated according to the procedure outlined by ***Johnson* et al. *(1955***) thus: **PCV =** $\frac{\delta ph}{\bar{x}}$ **x 100**, **GCV =** $\frac{\delta g}{\bar{x}}$ **x 100,** where $\bar{x}$ = General mean. The genetic advance expected under selection, assuming the selection intensity of 5% was calculated as suggested by ***Allard (1960***): $GA = {}^{K}\frac{\delta^2 g}{\delta^2 ph}\sqrt{\delta ph}$

where: GA= Expected genetic advance K= selection differential (2.06 at 5% selection intensity) and δph = phenotypic standard deviation. Genetic advance as percent of mean (GA%) was calculated using the formula: GA% = $\frac{GA}{\bar{x}}$ x 100

SDS PAGE

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was performed according to the method of ***Laemmli (1970***) to discriminate and fingerprint the twenty five faba bean genotypes irrigated with salt water. Total protein was randomly extracted from young leaves and the concentration of extracted protein was estimated using Biuret method and finally equal amount of protein was loaded on 12% acrylamide resolving gel for resolution of polypeptides. The gel was electrophoresed at 200v followed by staining with Commassie Brilliant blue R-250 for 2–3 h and then was destained in a solution of methanol and water (1:1) and 10% acetic acid. Gel was photographed and scored using gel documentation system 35 mm color film (200 ASA) and scanned with Bio-Rad video densitometer model 620 USA, at a wavelength of 577. Software data was analyzed for Bio-Rad Model 620 densitometer and computer were used as illustrated by the manufacturer. For SDS-PAGE the presence of each band was also scored as 1 and its absence as 0. The bands were designated on the basis of Protein Molecular Weight Marker (PMW). Band size was estimated by comparing with 1-kb ladder (Invitrogen, USA) using Total lab, TL120 1D v2009 (nonlinear Dynamics Ltd, USA). The binary data matrices were entered into the NTSYSpc (Ver. 2.1) and analyzed using qualitative routine to generate similarity coefficient and used to construct a dendrogram using un-weighted pair group method with arithmetic average (UPGMA) and sequential hierarchical and nested clustering (SHAN) routine (***Nei, 1973 and 1978***).

DNA extraction

Two-week-old faba bean leaves from selected accessions (Table 1) were collected, dropped in liquid N_2 and stored at -80ºC until DNA isolation. DNA isolation was carried out using modified SDS protocol (***Milligan, 1998***).

The quality and concentration of extracted DNA was detected using 0.8% agarose gel electrophoresis and spectrophotometer. Dilutions with TE were carried out and the concentration was fixed at 100 ng ml^{-1}.

Randomly amplified polymorphic DNA (RAPD) analysis

Three primers for RAPD were used in the study and were successful in generating reproducible and reliable amplicons for the twenty five faba bean genotype. Names and sequences of the selected primers are shown in Table (3). The amplification reaction was carried out in 25 µl reaction volume containing 1x PCR buffer, 4 mM $MgCl_2$, 0.2 mM dNTPs, 20 pmole of each primer, 2 units Taq DNA polymerase and 25 ng template DNA. PCR amplification was performed in a Perkin Elmer 2400 thermocycler (Germany), programmed to fulfill 40 cycles after an initial denaturation cycle for 4 min at 94ºC. Each cycle consisted of a denaturation step at 94ºC for 1 min, an annealing step at 40ºC for 2 min, and an extension step at 72ºC for 2 min, followed by an extension cycle for 7 min at 72ºC.

Detection of PCR Products

The products of RAPD-based PCR analyses were detected using agarose gel electrophoresis (1.2% in 1X TBE buffer), stained with ethidium bromide (0.3 ug/ml) and then visually examined with UV trans-illuminator and photographed using a CCD camera (UVP, UK).

Table 3. Names of primers and their nucleotide sequences used in DNA profile.

No.	Name	Sequence
1	OP-A07	5'GAAACGGGTG 3'
2	OP-B20	5' GGACCCTTAC 3'
3	OP-F09	5' CCAAGCTTCC 3'

Data analysis

Clear, un-ambiguous and reproducible bands were considered for scoring. Each band was considered a single locus. Data were scored as (1) for the presence and (0) for the absence of a given DNA band. Band size was estimated by comparing with 1-kb ladder (Invitrogen, USA) using Total lab, TL120 1D v2009 (nonlinear Dynamics Ltd, USA). The binary data matrices were entered into the NTSYSpc (Ver. 2.1) and analyzed using qualitative routine to generate similarity coefficient and used to construct a dendrogram using un-weighted pair group method with arithmetic average (UPGMA) and sequential hierarchical and nested clustering (SHAN) routine (Nei, 1973 and 1978).

RESULTS AND DISCUSSION

Analysis of variance during both seasons is presents in Table (4), which showed highly significant ($p \leq 0.01$) differences among genotypes for all characteristics, while differences among replicates were non-significant except for No. of seeds plant^{-1} and seed yield plant^{-1} in the first season, which were highly significant ($p \leq 0.01$) and significant ($p \leq 0.05$), respectively.

Mean performance of faba bean parents and lines during 2015/16 and 2016/17 seasons, as well as, the mean of both seasons is presented in Tables (5 and 6). Data revealed that parents significantly differed for all traits in both seasons, except for plant height in the first season and No. of branches plant^{-1} in the second season, whereas, all lines significantly differed for all traits in both seasons. The mean of both seasons showed that Nubaria 1 (P2) possessed the lowest No. of pods and seeds plant^{-1} and seed yield plant^{-1} (6.32, 15.91 and 12.55 g., respectively), but it had the highest 100-seed weight (84.2 g), whereas Giza 843 (P3) recorded the highest No. of pods, seeds plant^{-1} and seed yield plant^{-1} (16.4, 47.7 and 41.3 g., respectively). From these data we can conclude that Nubaria 1 (P2) is susceptible variety to salinity, while Giza 843 (P3) is moderately tolerant. These results are in agreement with those obtained by ***Abdelraouf et* al. *(2016***) and ***Abdel-Salam (2017***). Variety Sakha 3 (P4) recorded the lowest 100-seed weight (65.3 g).

Regarding the lines over the two seasons, line M6 possessed the highest mean for the both seasons for No. of branches, No. of pods, No. of seeds plant^{-1} and seed yield plant^{-1} (4.47, 23.64, 65.60 and 44.70 g., respectively), while Line H3 recorded the shortest plant height (81.6 cm.), and Line M4 possessed the tallest plant height (102.0 cm.). However, Line H9 exhibited the lowest mean of No. of branches plant^{-1} (3.20) and 100-seed weight (64.9 g), Line M7 had the lowest mean of No. of pods plant^{-1} (10.8) and seed yield plant^{-1} (19.4 g) and Line M9 showed the lowest mean of No. of seeds plant^{-1} (23.9). On the other hand, Line H5 had the highest mean of 100-seed weight (83.0 g). For plant height, No. of pods, seeds plant^{-1} and 100-seed weight possessed high mean value in the second season as compared to the first one because the EC dS m^{-1} of the soil in the first season (6.67) was higher than that in the second season (4.35) as shown

in Table (1). These results are in agreement with those obtained by ***Abdelraouf,* et al. *(2016***) who reported that increasing salt concentration decreased the fresh and dry weight of shoots and roots, shoot height and leaf area.

Nevertheless, genotype No. 21 (M6) possessed the highest mean value for No. of pods plant^{-1} (36.8) in the second season and No. of seeds plant^{-1} (29.2 and 102.0) in both seasons, respectively. On the other hand, genotype No. 2 (Nubaria 1) recorded the lowest mean value for plant height (91.0 cm.), No. of branches plant^{-1} (2.40) and seed yield plant^{-1} (13.42 g.) in the second season as well as it possessed the lowest mean value for No. of pods and seeds plant^{-1} (4.03 and 8.60) in the first season and (11.42 and 20.40) in the second one, respectively, while it had the highest mean value for 100-seed weight (102.5 g) in the first season. The tallest plant height was recorded in genotype No. 23 (M8) (85.2 cm) in the first season and genotype No.19 (M4) in the second one (129.0 cm.), whereas, genotype No. 3 (Giza 843) exhibited the shortest plant height (54.0 cm.) in the first season. Genotype No.12 (H7) and genotype No.5 (Giza 716) showed the highest No. of branches plant^{-1} (4.57 and 6.00) in the first and second season, respectively. On the other hand, genotype No. 20 (M5) had the lowest mean value for No. of branches plant^{-1} (3.21) in the first season. Genotype No.15 (H10) recorded the highest mean value for No. of pods plant^{-1} (11.76) in the first season.

The highest mean values for seed yield plant^{-1} were recorded in genotype No. 10 (H5) (20.2 g) and genotype No.6 (H1) (74.5) in the first season and second one, respectively. Meanwhile genotype No. 22 (M7) had the lowest seed yield (8.81 g) in the first season. On the other hand, the lowest mean values for 100-seed weight (g) were recorded in genotype No.14 (H9) and genotype No. 4 (Sakha 3) (59.2 and 51.9) in the first season and second one, respectively, whereas, genotype Misr 3 had the highest mean value (98.0) in the second season.

Table 4. Mean square for yield and its components of faba bean genotypes under salinity stress during two seasons 2015/16 and 2016/17.

S.O.V.	d.f.	Plant height (cm.)		No. of branches plant^{-1}		No. of pods plant^{-1}	
		1st	2nd	1st	2nd	1st	2nd
Reps.	4	16.00	12.95	0.27	0.092	1.23	0.59
Genotype.	24	396.80**	534.80**	0.69**	2.56**	22.20**	201.96**
Error	96	15.90	13.36	0.22	0.219	0.59	5.58
S.O.V	d.f.	No. of seeds plant^{-1}		Seed yield plant^{-1} (g)		100-seed weight (g)	
		1st	2nd	1st	2nd	1st	2nd
Reps.	4	4.97**	33.35	2.10*	11.41	19.31	2.38
Genotype	24	138.12**	1796.09**	54.77**	1373.12**	385.81**	587.49**
Error	96	1.29	36.90	0.84	23.00	7.92	7.95

***and **: Significant at 0.05 and 0.01 level of probability, respectively.**

Table 5. Mean performance of plant height, No. of branches plant^{-1} and No. of pods plant^{-1} under salinity stress of faba bean genotypes during two seasons 2015/16 and 2016/17.

No.	Characteristic / Genotype	Plant height (cm.)			No. of branches plant^{-1}			No. of pods plant^{-1}		
		1st	2nd	Mean	1st	2nd	Mean	1st	2nd	Mean
1	Misr 3 (P1)	59.50	97.00	78.25	3.40	3.70	3.55	5.60	20.10	12.85
2	Nubaria 1 (P2)	55.58	91.00	73.29	3.95	2.40	3.18	4.03	8.60	6.32
3	Giza 843 (P3)	54.00	93.80	73.90	3.50	3.00	3.25	10.60	22.20	16.40
4	Sakha 3 (P4)	60.00	92.00	76.00	4.23	4.00	4.12	5.63	17.00	11.32
5	Giza 716 (P5)	54.67	96.00	75.34	4.12	6.00	5.06	5.38	18.50	11.94
L.S.D 0.05		**NS**	**4.00**	**-**	**0.65**	**NS**	**-**	**2.13**	**0.97**	**-**
6	H1	60.50	108.00	84.25	3.93	4.50	4.22	6.03	36.00	21.02
7	H2	69.67	109.40	89.54	3.70	2.90	3.30	6.70	23.00	14.85
8	H3	61.17	102.00	81.59	3.87	3.33	3.60	10.53	26.60	18.57
9	H4	65.00	106.80	85.90	4.10	3.93	4.02	10.20	24.33	17.27
10	H5	77.33	103.00	90.17	3.73	3.77	3.75	9.47	22.13	15.80
11	H6	70.00	104.20	87.10	3.40	3.60	3.50	5.37	23.17	14.27
12	H7	75.58	99.00	87.29	4.57	2.90	3.74	7.60	25.27	16.44
13	H8	73.00	92.00	82.50	3.80	3.33	3.57	7.40	22.23	14.82
14	H9	65.50	107.60	86.55	3.27	3.13	3.20	8.17	22.73	15.45
15	H10	72.90	104.00	88.45	3.98	3.70	3.84	11.76	24.83	18.30
16	M1	74.42	109.00	91.71	3.42	3.45	3.44	8.80	25.07	16.94
17	M2	81.67	121.00	101.34	3.40	4.13	3.77	6.67	29.80	18.24
18	M3	73.42	112.00	92.71	3.68	4.00	3.84	6.32	25.10	15.71
19	M4	75.03	129.00	102.02	3.59	4.07	3.83	9.70	29.57	19.64
20	M5	78.90	118.00	98.45	3.21	3.90	3.56	9.24	15.60	12.42
21	M6	76.33	124.00	100.17	4.13	4.80	4.47	10.47	36.80	23.64
22	M7	76.17	113.00	94.59	3.05	4.10	3.58	6.18	15.40	10.79
23	M8	85.17	116.00	100.59	4.20	4.30	4.25	8.23	21.10	14.67
24	M9	76.93	117.00	96.97	3.67	4.00	3.84	6.47	16.20	11.34
25	M10	77.17	108.00	92.59	3.98	3.80	3.89	10.07	15.60	12.84
LSD 0.05		**5.27**	**4.13**	**-**	**0.57**	**0.55**	**-**	**0.98**	**3.05**	**-**
General LSD 0.05		**5.02**	**4.60**	**-**	**0.59**	**0.59**	**-**	**0.97**	**2.98**	**-**
CV%		**5.70**	**3.42**	**-**	**12.46**	**12.35**	**-**	**9.74**	**10.42**	**-**

The promising line M6 produced the highest mean value for No. of branches plant^{-1} (4.13 and 4.80), No. of pods plant^{-1} (10.47 and 36.80), No. of seeds plant^{-1} (29.20 and 102.00) and seed yield plant^{-1} (18.58 and 70.82 g.) in the first and second season, respectively, while variety Giza 843 possessed the highest mean value for No. of pods plant^{-1} (10.60 and 22.20), No. of seeds plant^{-1} (24.5 and 70.8) and seed yield plant^{-1} (17.6 and 64.9 g) in the first and second seasons, respectively. Therefore, promising line M6 could be used in comparative yield trials to be released as a new cultivar, which possesses high yielding under salinity condition.

Table 6. Mean performance of faba bean genotypes for No. of seeds plant^{-1}, Seed yield plant^{-1} (g) and 100-seed weight (g) under salinity stress during two seasons 2015/16 and 2016/17.

No.	Characteristic / Genotype	No. of seeds plant^{-1}			Seed yield plant^{-1} (g)			100-seed weight (g)		
		1st	2nd	Mean	1st	2nd	Mean	1st	2nd	Mean
1	Misr 3 (P1)	16.30	50.40	33.35	11.17	49.44	30.31	68.45	98.00	83.23
2	Nubaria 1 (P2)	11.42	20.40	15.91	11.68	13.42	12.55	102.48	65.87	84.18
3	Giza 843 (P3)	24.50	70.80	47.65	17.56	64.94	41.25	71.62	91.84	81.73
4	Sakha 3 (P4)	16.93	43.40	30.17	13.33	22.40	17.87	78.64	51.90	65.27
5	Giza 716 (P5)	14.70	47.80	31.25	11.13	43.58	27.36	75.65	91.21	83.43
LSD 0.05		3.66	1.12	-	2.37	1.27	-	4.03	4.14	-
6	H1	13.53	97.70	55.62	9.97	74.50	42.24	73.65	76.26	74.96
7	H2	14.87	62.70	38.79	10.26	44.84	27.55	69.06	71.50	70.28
8	H3	25.20	73.70	49.45	17.90	63.15	40.53	71.04	85.67	78.36
9	H4	27.73	66.53	47.13	18.30	57.27	37.79	65.84	86.11	75.98
10	H5	26.53	62.52	44.53	20.19	55.49	37.84	77.33	88.76	83.05
11	H6	14.23	64.48	39.36	11.12	48.76	29.94	78.30	75.58	76.94
12	H7	18.65	69.97	44.31	14.33	56.32	35.33	76.81	80.50	78.66
13	H8	20.20	65.60	42.90	12.58	61.62	37.10	62.45	93.94	78.20
14	H9	20.67	61.80	41.24	12.21	43.71	27.96	59.15	70.70	64.93
15	H10	28.89	60.80	44.85	18.81	46.82	32.82	65.17	77.03	71.10
16	M1	21.77	64.80	43.29	14.37	52.13	33.25	66.00	80.42	73.21
17	M2	15.13	75.53	45.33	10.81	67.50	39.16	71.49	89.34	80.42
18	M3	14.77	70.17	42.47	11.42	54.01	32.72	77.37	76.95	77.16
19	M4	21.27	86.10	53.69	14.14	73.82	43.98	66.44	85.73	76.09
20	M5	24.96	44.80	34.88	17.89	32.80	25.35	78.90	73.17	76.04
21	M6	29.20	102.00	65.60	18.58	70.82	44.70	63.63	69.45	66.54
22	M7	14.13	43.80	28.97	8.81	29.89	19.35	62.44	68.23	65.34
23	M8	19.63	49.20	34.42	12.48	38.96	25.72	63.71	80.35	72.03
24	M9	17.10	30.80	23.95	11.20	27.68	19.44	65.50	90.43	77.97
25	M10	19.48	41.20	30.34	14.27	27.59	20.93	73.33	66.89	70.11
LSD 0.05		1.47	8.36	-	1.13	6.62	-	3.47	3.47	-
General LSD 0.05		1.43	7.65	-	1.16	6.04	-	3.55	3.55	-
CV%		5.78	9.95	-	6.67	9.82	-	3.94	3.55	--

The relation between seed yield and its attributes under salinity stress for two seasons 2015/16 and 2016/17 is shown in Table (7). The correlation coefficient confirmed the highly significant positive correlation (0.98**) between both of No. of pods plant^{-1} and seed yield plant^{-1} with No. of seeds plant^{-1}. Also there was highly significant positive correlation (0.96**) between No. of pods plant^{-1} and seed yield plant^{-1}. Non-significant positive correlations were found between plant heights, seed yield plant^{-1} and 100-seed weight with No. of branches plant^{-1} (0.09, 0.012 and 0.11, respectively), while negative correlation between No. of pods plant^{-1} and 100-seed weight was highly significant (-0.19**). These results confirmed that No. of pods, seeds plant^{-1} and seed yield plant^{-1} are considered as good criteria for selection for salinity tolerance.

Table 7. Relation between seed yield and its attributes under salinity stress during two seasons 2015/16 and 2016/17.

	No. of branches plant^{-1}	No. of pods plant^{-1}	No. of seeds plant^{-1}	Seed yield plant^{-1} (g)	100-seed weight(g)
Plant height (cm.)	0.09	0.81**	0.79**	0.70**	0.26**
No. of branches plant^{-1}	-	0.17**	0.13*	0.12	0.11
No. of pods plant^{-1}	-	-	0.98**	0.96**	-0.19**
No. of seeds plant^{-1}	-	-	-	0.98**	0.34**
Seed yield plant^{-1} (g)	-	-	-	-	0.50**

The variations among genotypes were mostly due to the genetic makeup or structure factors rather than environmental ones, as indicated by higher genetic variance, which indicated that these traits were genetically controlled and are less influenced by the environment. The estimates of genotypic variance (δ^2g), phenotypic variance (δ^2ph), broad-sense heritability (H^2), genotypic coefficient of variation (G.C.V.) and phenotypic coefficient of variation (P.C.V.) as well as expected genetic advance under 5% selection intensity as percentage of the general mean (GA%) are presented in Tables (8 and 9). The phenotypic variance (δ^2ph) was greater than the genotypic variance (δ^2g) for all studied traits in both seasons. The genotypic variance (δ^2g) and phenotypic variance (δ^2ph) values for plant height were the highest values (92.1 and 76.2 cm) in the first season, as well as, No. of seeds plant^{-1} possessed the highest values (388.7 and 351.8) in the second season for phenotypic and genotypic variance, respectively. The estimated value of heritability in broad sense was high for all characteristics in both seasons except for No. of branches plant^{-1} in the first season was low (30.0). The high heritability indicated that selection based on mean values would be successful in improving these traits.

G.C.V. and P.C.V. estimates were generally different for all studied traits in both seasons as they ranged from 8.2 to 33.6 for G.C.V. and from 10.2 to 35.0 for P.C.V. The high G.C.V. recorded by the above-mentioned traits alone is not sufficient for the determination of the extent of the advance to be expected by selection. ***Burton (1952***) suggested that G.C.V. together with heritability estimates would give the best picture of the extent of the advance to be expected by selection. Comparatively, the highest genetic advance was recorded for all characteristics under study except for plant height in the second season (18.5%) and No. of branches plant^{-1} in the first season (9.2%). The magnitude of heritability in broad sense was low coupled with low genetic advance for plant height in the second season.

In the present study, it was obvious from Tables (8 and 9), that characteristics with high G.C.V. possessed high GA percent and vice versa irrespective of the heritability estimates indicating the importance of G.C.V. The high estimates of G.C.V., H^2 and GA% were observed for No. of seeds plant^{-1} and seed yield plant^{-1} in the second season, which suggests the predominance of additive gene effects and selection would be useful for the improvement of these characteristics. Similar results have also been reported by ***Dar and Sharma (2011)*** and ***Mahmoud and Ghareeb (2015***). The low values of expected genetic advance for some characteristics were due to low variability for the traits indicated by the medium G.C.V. and P.C.V. values. Therefore, even if heritability estimates provide basis for selection on phenotypic performance, the estimates of heritability and genetic advance should always be considered

simultaneously, as high heritability is not always associated with high genetic advance. These results are in agreement with those obtained by ***Bakhiet* et al.** ***(2015***) and ***Abd El-Mohsen,* et al. *(2016***). Presence of genetic variability and heritability estimates would be helpful to the breeder to estimate genetic advance and to predict percentage of genetic advance in the population under study. Success of genetic improvement is attributed to the magnitude and nature of variability present for specific characteristic. Accordingly all the agronomic characteristics are considered for analysis showed high heritability, constituting high breeding value, which has more additive genetic effects that is important for crop improvement. Similar results were reported by ***El-Hosary and Nawar (1984), Mahmoud and Al-Ayobi (1986) and Mulualem* et al. *(2013).***

Table 8. Estimation of heritability, genetic advance, G.C.V and P.C.V for plant height, No. of branches plant^{-1} and No. of pods plant^{-1} of 25 faba bean genotypes at two seasons 2015/2016 and 2016/2017.

	Plant height (cm.)		No. of branches plant^{-1}		No. of pods plant^{-1}	
	1st	2nd	1st	2nd	1st	2nd
$\delta^2 g$	76.18	104.29	0.09	0.47	4.32	39.27
$\delta^2 ph$	92.08	117.65	0.31	0.69	4.91	44.86
h^2_b	82.74	88.64	30.01	68.10	88.04	87.56
G.C.V	12.47	9.55	8.16	18.05	26.44	27.64
P.C.V	13.71	10.15	14.89	21.87	28.18	29.53
G.A	16.35	106.91	0.35	1.16	4.02	12.08
G.A%	23.37	18.53	9.21	30.68	51.10	53.27

Table 9. Estimation of heritability, genetic advance, G.C.A and P.C.V for No. of seeds plant^{-1}, seed yield plant^{-1} (g) and 100-seed weight (g) of 25 faba bean genotypes at two seasons 2015/2016 and 2016/2017.

	No. of seeds plant^{-1}		Seed yield plant^{-1} (g)		100-seed weight (g)	
	1st	2nd	1st	2nd	1st	2nd
$\delta^2 g$	27.36	351.84	10.79	270.02	27.36	351.84
$\delta^2 ph$	28.66	388.74	11.63	293.02	28.66	388.74
h^2_b	95.48	90.51	92.74	92.15	95.48	90.51
G.C.V	26.59	30.71	23.83	33.63	26.59	30.71
P.C.V	27.21	32.28	24.75	35.04	27.21	32.28
G.A	10.53	36.76	6.52	32.50	10.53	36.76
G.A%	53.53	60.18	47.28	66.51	53.53	60.18

Fingerprinting based on proteins.

The electrophoretic banding patterns of proteins extracted from the leaves of the twenty five faba bean genotypes are shown in **Fig.'s 1 and 2.** The presence and absence of bands were represented with (1) and (0), respectively. The results of SDS-PAGE revealed a total number of

19 bands with molecular weights (MW) ranging from about 10.2 to 173.0 kDa, which were not necessarily present in all 25 genotypes. Data revealed two common bands (monomorphic), while the remaining 17 bands were polymorphic with 89.9% polymorphism. The twenty-five genotypes showed different patterns in presence of bands (Fig.'s. 1, 2).

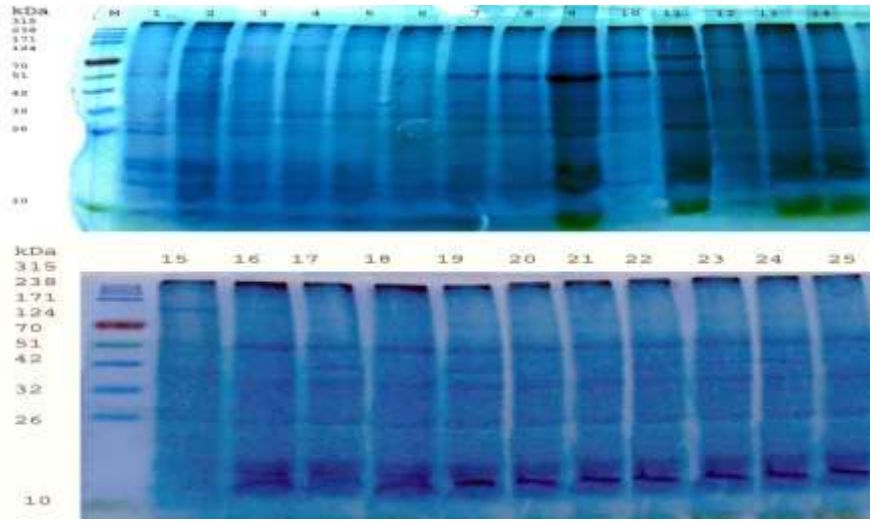


Fig. 1. SDS-PAGE of protein banding patterns of twenty five faba bean genotypes where (1) Misr 3, (2) Nubaria1, (3) Giza 843, (4) Sakha 3, (5) Giza 716, (6) H1, (7) H2, (8) H3, (9) H4, (10) H5, (11) H6, (12) H7, (13) H8, (14) H9. (15) H 10, (16) M1, (17) M2, (18) M3, (19) M4, (20) M5, (21) M6, (22) M7, (23) M8, (24) M9, and (25) M10.

Minimum number of bands (3) was present in Giza 843, which is characterized by moderate tolerance to salinity and relatively high seed yield per plant with 100-seed weight per plant. The maximum number of bands (7) was found in H5, which tolerates the salinity and has relatively high number of seeds per plant with significant increase in 100-seed weight per plant (83.0 g). However, there were resemblance between many genotypes, especially in band number 14 like M4 and M6.

Genotype M6 contained 4 polymorphic bands and is tolerant to salinity with high grain yield per plant, plant height reached 100.2 cm with significant increase in number of branches per plant (4.47) and significant increase in number of pods per plant (23.64), while others were characterized by a unique fingerprinting. At the same time, there were three marker bands for three moderate sensitive genotypes; band number 1 at MW 173 kDa, band number 2 with MW 155.1 and band 16 with MW of 13.4 that uniquely characterized H_4, H_{10} and Sakha 3, respectively. However, there was no negative marker bands appeared. These results agreed with ***Hughes and Murphy (1983), Afify and Shousha (1988) and Abdel-Tawab*** **et al.** ***(1993***) who confirmed that SDS-PAGE was a highly successful technique in genotype identification. Protein marker confirmed the use of electrophoretic analysis of seed storage protein of *Vicia faba* L. as an aid to genotype identification as reported by ***Goodrich*** **et al.** ***(1985***).

Potokina and Eggi (1997) concluded that protein electrophoregram may be useful for identifying *Vicia angustifolia* accession of Asiatic origin. ***Kasarada* et al. *(1998) and Jaramillo* et al. *(1999***) reported that SDS-PAGE was widely used to separate proteins, which are directly related to genetic background and can be used to certify the genetic makeup of wild, genotypes, or newly derived cereal plants.

Genetic similarities between the twenty five faba bean genotypes based on protein pattern analysis is shown in Table (10). The highest similarity index recorded was (1.0), between genotype M6 and genotype M1, while the lowest similarity index recorded was zero, which appeared 116 times from total of 300 value with percentage of 38.7%, which reflects high level of dissimilarity between genotypes used in these study.

Table 10. Similarity coefficients (Jaccard Similarity Measure) among the studied *Vicia faba* L. genotypes based on protein banding patterns, where G1= Misr 3, G2= Nubaria1, G3= Giza 843, G4= Sakha 3, G5= Giza 716, G6= H1, G7= H2, G8= H3, G9= H4, G10= H5, G11= H6, G12= H7, G13= H8, G14= H9. G15= H 10, G16= M1, G17= M2, G18= M3, G19= M4, G20= M5, G21= M6, G22= M7, G23= M8, G24= M9, and G25 = M10..

G.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
1	1																								
2	0.25	1																							
3	0.60	0.14	1																						
4	0.11	0.25	0.0	1																					
5	0.25	0.25	0.0	0.25	1																				
6	0.14	0.14	0.0	0.14	0.33	1																			
7	0.42	0.11	0.33	0.25	0.25	0.33	1																		
8	0.42	0.25	0.14	0.11	0.25	0.33	0.25	1																	
9	0.22	0.37	0.28	0.22	0.10	0.0	0.22	0.1	1																
10	0.11	0.42	0.0	0.25	0.25	0.33	0.11	0.42	0.10	1															
11	0.10	0.10	0.12	0.0	0.0	0.0	0.10	0.0	0.09	0.0	1														
12	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.10	1													
13	0.0	0.12	0.0	0.0	0.0	0.0	0.0	0.12	0.0	0.12	0.11	0.12	1												
14	0.09	0.09	0.11	0.0	0.09	0.0	0.0	0.0	0.08	0.0	0.18	0.09	0.22	1											
15	0.12	0.5	0.0	0.12	0.12	0.16	0.12	0.28	0.11	0.28	0.11	0.0	0.14	0.0	1										
16	0.0	0.12	0.0	0.0	0.12	0.0	0.0	0.0	0.11	0.0	0.25	0.0	0.0	0.22	0.14	1									
17	0.11	0.0	0.14	0.0	0.0	0.0	0.11	0.11	0.22	0.11	0.0	0.11	0.0	0.09	0.0	0.12	1								
18	0.14	0.0	0.20	0.0	0.0	0.0	0.14	0.14	0.12	0.14	0.0	0.0	0.0	0.11	0.0	0.16	0.33	1							
19	0.25	0.0	0.33	0.0	0.11	0.0	0.25	0.11	0.22	0.0	0.10	0.0	0.0	0.09	0.0	0.12	0.25	0.14	1						
20	0.12	0.0	0.16	0.0	0.12	0.0	0.12	0.12	0.11	0.0	0.0	0.0	0.0	0.22	0.0	0.33	0.28	0.40	0.50	1					
21	0.0	0.12	0.0	0.0	0.12	0.0	0.0	0.0	0.11	0.0	0.25	0.0	0.0	0.22	0.14	1	0.12	0.16	0.12	0.33	1				
22	0.0	0.1	0.0	0.0	0.22	0.12	0.10	0.0	0.09	0.0	0.33	0.10	0.11	0.30	0.11	0.06	0.10	0.12	0.10	0.25	0.06	1			
23	0.0	0.22	0.0	0.0	0.10	0.12	0.10	0.10	0.09	0.22	0.09	0.0	0.25	0.08	0.25	0.11	0.10	0.12	0.0	0.0	0.11	0.20	1		
24	0.0	0.22	0.0	0.22	0.10	0.0	0.0	0.10	0.09	0.37	0.0	0.0	0.25	0.08	0.11	0.0	0.10	0.12	0.0	0.0	0.0	0.0	0.5	1	
25	0.0	0.25	0.0	0.11	0.11	0.14	0.0	0.25	0.22	0.42	0.0	0.0	0.12	0.0	0.12	0.0	0.11	0.0	0.11	0.0	0.0	0.0	0.1	0.22	1

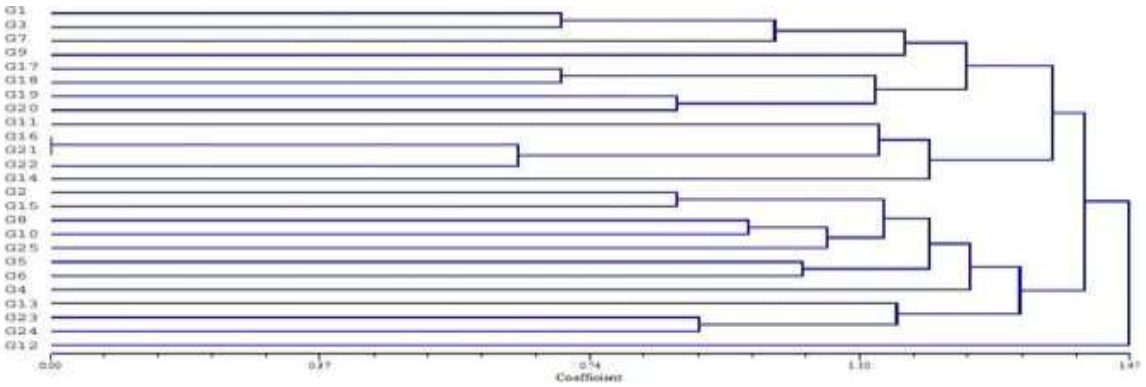


Fig. 2. A Linkage dendrogram of studied *Vicia faba* L. genotypes based on analysis of protein banding pattern, where G1= Misr 3, G2= Nubaria1, G3= Giza 843, G4= Sakha 3, G5= Giza 716, G6= H1, G7= H2, G8= H3, G9= H4, G10= H5, G11= H6, G12= H7, G13= H8, G14= H9. G15= H 10, G16= M1, G17= M2, G18= M3, G19= M4, G20= M5, G21= M6, G22= M7, G23= M8, G24= M9, and G25 = M10.

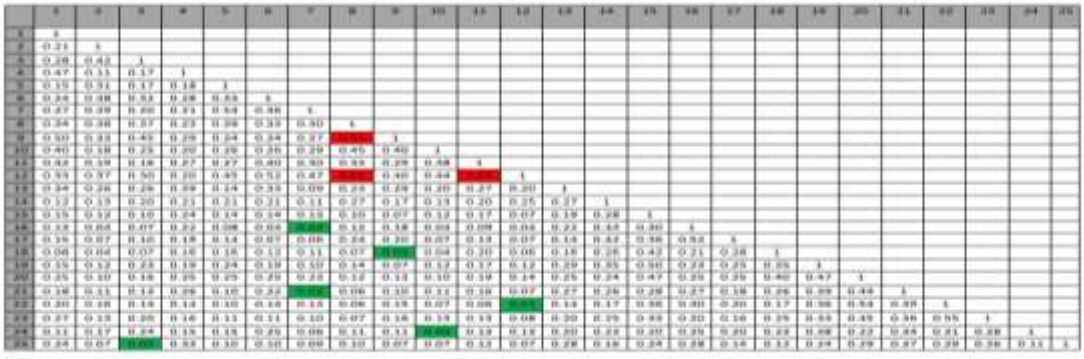
Plants frequently encounter external stress conditions, which may cause harmful effects on the growth and productivity. These stresses can be either abiotic or biotic. Abiotic stress as salts in irrigation water or soil inhibit plant growth, affect crop yield and sometimes quality as salt reduces water uptake. Excessive salt in the root zone can cause further reductions in growth and yield because of specific toxic ion effects (***Qadir and Oster, 2004***). The inherent ability to tolerate or resist root-zone salinity depends on crops and their cultivars (***Maas and Grattan, 1999; Shannon and Grieve, 1999 and Rameshwaran*** **et al.,** ***2015.)***

Table 11. Number of monomorphic fragments, polymorphic fragments and percentage of polymorphism obtained per RAPD primers for 25 genotypes under investigation.

Primers	Sequence	Total No. of fragments	Total number of bands	polymorphic fragments	Polymorphism %
OP-A07	5'GAAACGGGTG 3'	133	22	22	100 %
OP-B20	5' GGACCCTTAC 3'	114	16	16	100 %
OP-F09	5' CCAAGCTTCC 3'	123	21	21	100 %
Total		**370**	**59**	**59**	**100 %**

There were highly similarity between genotypes 8 and 9 based on RAPD-PCR banding patterns (Table 12), the same similarity was found based on seed yield and its components (Tables 5 and 6). **In addition,** there were high similarity coefficient between genotype 12 with both 8 and 11 genotypes. Meanwhile, there were low similarity coefficient between 3 with 25, 9 with 18, 10 with 24, 12 with 22 and 7 with both 16 and 21 genotypes.

Table 12. Similarity coefficients (Jaccard Similarity Measure) among the studied Vicia faba L. genotypes based on RAPD-PCR banding patterns, , where G1= Misr 3, G2= Nubaria1, G3= Giza 843, G4= Sakha 3, G5= Giza 716, G6= H1, G7= H2, G8= H3, G9= H4, G10= H5, G11= H6, G12= H7, G13= H8, G14= H9. G15= H 10, G16= M1, G17= M2, G18= M3, G19= M4, G20= M5, G21= M6, G22= M7, G23= M8, G24= M9, and G25 = M10.



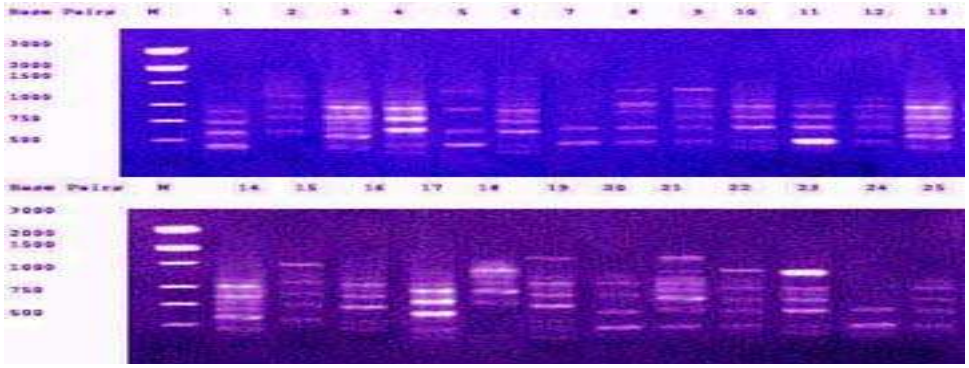


Fig. 3. DNA polymorphism of the twelve Faba bean genotypes amplified with the primer OP-A07, where 1= Misr 3, 2= Nubaria1, 3= Giza 843, 4= Sakha 3, 5= Giza 716, 6= H1, 7= H2, 8= H3, 9= H4, 10= H5, 11= H6, 12= H7, 13= H8, 14= H9. 15= H 10, 16= M1, 17= M2, 18= M3, 19= M4, 20= M5, 21= M6, 22= M7, 23= M8, 24= M9, and 25= M10.

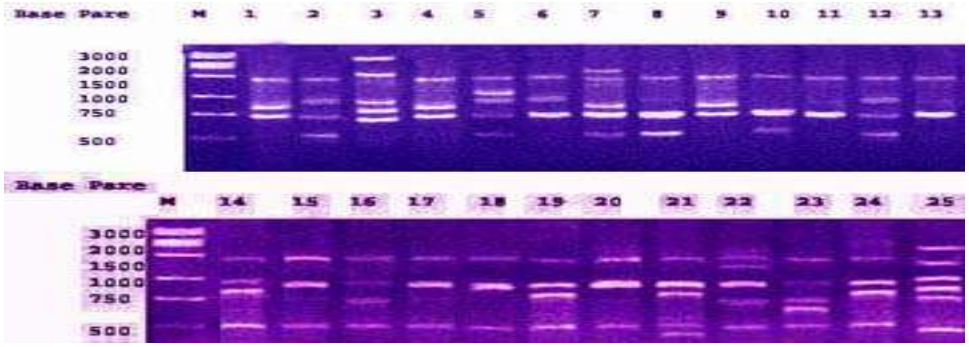


Fig. 4. DNA polymorphism of the twelve Faba bean genotypes amplified with the primer OP-B20, where 1= Misr 3, 2= Nubaria1, 3= Giza 843, 4= Sakha 3, 5= Giza 716, 6= H1, 7= H2, 8= H3, 9= H4, 10= H5, 11= H6, 12= H7, 13= H8, 14= H9. 15= H 10, 16= M1, 17= M2, 18= M3, 19= M4, 20= M5, 21= M6, 22= M7, 23= M8, 24= M9, and 25= M10.

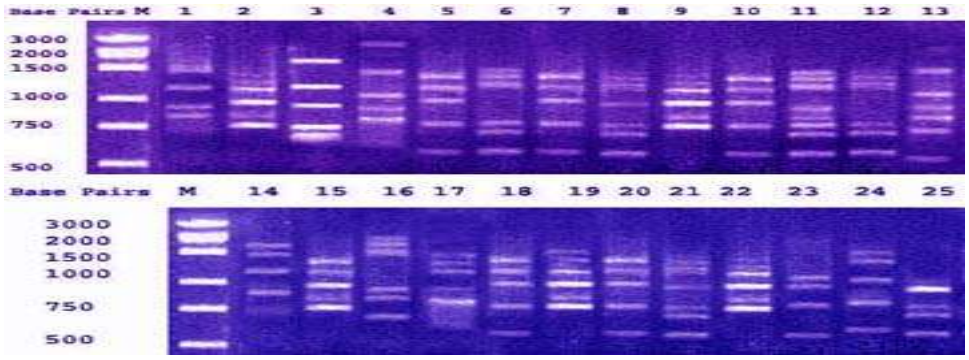


Fig. 5. DNA polymorphism of the twelve Faba bean genotypes amplified with the primer OP-F09, where 1= Misr 3, 2= Nubaria1, 3= Giza 843, 4= Sakha 3, 5= Giza 716, 6= H1, 7= H2, 8= H3, 9= H4, 10= H5, 11= H6, 12= H7, 13= H8, 14= H9. 15= H 10, 16= M1, 17= M2, 18= M3, 19= M4, 20= M5, 21= M6, 22= M7, 23= M8, 24= M9, and 25= M10.

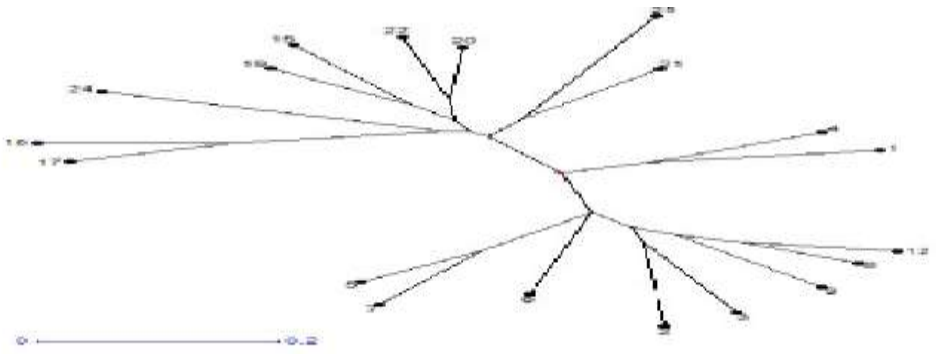


Fig. 6. A Linkage dendrogram of studied *Vicia faba* L. based on the analysis of RAPD-PCR banding patterns.

Fingerprinting based on RAPD.

From a total of 12 primers screened initially, 3 primers responded with 59 reproducible bands. A total of 370 bands were detected in all the twenty five genotypes using the 3 primers as shown in Table (11) and Fig.'s (3, 4 and 5). The number of amplified bands ranged from 16 (OPB-20) to 22 (OPA-07). Out of 59 bands amplified, 59 bands were polymorphic with an average of 19.67 polymorphic bands per primer. The percentage polymorphism was found to be 100%. A dendrogram grouped the 25 genotypes of *Vicia faba* L. into three main clusters (Fig. 6), with Jaccard's similarity coefficient ranging from 0.02 to 0.56 (Table 12). Cluster I comprised genotypes: 2, 3, 5, 6, 7, 8, 9 and 12 in three sub clusters, while cluster II comprised genotypes; 15, 16, 17, 19, 20, 21, 22, 24 and 25 in three sub clusters. Cluster III consisted of two genotypes; 1 and 4.

Analysis of DNA polymorphism and identification of superior genotypes are the prerequisite of any breeding program. Knowledge of genetic variation and genetic relationship among diverse germplasms is an important consideration for classification, utilization of germplasm resources and breeding. It would not be possible to identify molecular markers, without determining the DNA polymorphism reliably. Information on the DNA polymorphism allows the organization of the variability in the germplasm, assisting parent selection and paving the road to genetic gains. Experiments with Broad bean (*Vicia faba* L.) have demonstrated the potential of RAPD markers as a rapid and efficient method for distinguishing among different genomes. In the present study, a total of 370 amplified products were obtained using 3 RAPD primers. The number of DNA fragments amplified ranged from 16 to 22. The Jaccard's similarity coefficient ranged from 0.02 to 0.56. No uniform clusters were obtained according to the locations of germplasm were collected from the dendrogram obtained across twenty five *Vicia faba* L. germplasms, which reflects little or no location specificity among *Vicia faba* L. germplasms, which agreed with the findings found in salinity tolerance in some *Vicia faba* L. genotypes (***Megahed* et al., *2015***), peanut (***Azzam* et al. *2007***), Pea (***Bagheri* et al., *1995***) and in pea (***Hoey* et al. *1996***). DNA

Polymorphism in a specified population of *Vicia faba* L. is due to the presence of genetic variants characterized by different alleles and their frequency of allocation in a population.

The UPGMA cluster analysis assumes a constant evolutionary rate among germplasm and is typically most appropriate for diversity study within species. Since, it is taxonomically not valid to assume equal rates of evolution among different species; this limitation may have influenced the present findings. The clustering of the genotypes showed that no uniform clusters were obtained in separate cluster, which differentiate genotypes 1 and 4. So, the observation presented here demonstrated the utility of RAPDs markers in segregating genetic variation among different twenty five genotypes of *Vicia faba* L. which helps in breeding programs and its conservation. The genotype specific diagnostic bands using RAPD markers affords a prospect to be converted into SCAR markers for the discrimination of these genotypes after further validation of those markers by using more number of genotypes.

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دراسات جزيئية ووراثية لبعض لسلالات مبشرة من الفول البلدي تحت ظروف الإجهاد الملحي فى منطقة النوبارية

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الملخص العربي

أجريت هذه الدراسة بمحطة البحوث الزراعية بالنوبارية – مركز البحوث الزراعية خلال موسمي ١٦/٢٠١٥ ، ١٧/٢٠١٦ لتقييم ٢٠ سلالة مبشرة من الفول البلدى وخمسة أصناف (مصر ٣ ، نوبارية ١ ، جيزة ٨٤٣ ، سخا ٣ ، جيزة ٧١٦) تحت تأثير الملوحة باستخدام تصميم القطاعات الكاملة العشوائية (RCBD) فى خمسة مكررات وكان EC التربة ٦,٦٧ (dSm^{-1}) فى الموسم الأول و ٤.٣٥ في الموسم الثاني. أعطت السلالة المبشرة رقم M6 أعلى متوسط لصفات عدد الأفرع/النبات (٤,١٣ ، ٤,٨٠) و عدد القرون/النبات (١٠,٤٧ ، ٣٦,٨٠) و عدد البذور/النبات (٢٩,٢٠ ، ١٠٢,٠٠) و محصول البذور/نبات (١٨,٥٨ ، ٧٠,٨٢ جم)، على التوالي مقارنة بما حققه الصنف جيزة ٨٤٣ لصفات عدد القرون/النبات (١٠,٦٠ ، ٢٢,٢٠) و عدد البذور/النبات (٢٤,٥٠ ، ٧٠,٨٠) و محصول البذور/النبات (١٧,٥٦ ، ٦٤,٩٤ جم) في موسمي الزراعة، على التوالي. وعلى لذلك يمكن التوصية بإدخال السلالة المبشرة رقم M6 ضمن سلسلة تجارب مقارنة المحصول لتأكيد النتائج لاعتمادها كصنف جديد يناسب الزراعة تحت الظروف الملحية. كما وجد أن هناك ارتباط عالي وموجب (٠.٩٨) بين كل من عدد القرون/النبات و محصول البذور/نبات مع عدد البذور/النبات. كانت قيمة المكافىء الوراثى عالية فى جميع الصفات في كلا الموسمين ماعدا صفة عدد الأفرع/النبات في الموسم الأول حيث كانت منخفضة (٣٠,٠١). أوضحت الدراسة أن القيم العالية للتقدم الوراثي بالانتخاب لمعظم الصفات تحت الدراسة مع كفاءة التوريث العالية تساعد المربي في انتخاب سلالات متحملة للملوحة. وكان التحسين الوراثي المتوقع من انتخاب أعلى ٥% من الصفات تحت الدراسة عاليا ماعدا صفة طول النبات فى الموسم الثانى (١٨,٥٣ سم) وصفة عدد الفروع/النبات فى الموسم الأول (٩,٢١). وقد كانت نتائج قيم المكافئ الوراثي عالية بالإضافة للتطور الوراثي العالي لصفات محصول البذور والصفات المرتبطة به وأن هذه الصفات يتحكم في توريثها الفعل الجيني المضيف. كما تم تحليل البروتين الكلى لجميع التراكيب الوراثية وتفريده بطريقة SDS-PAGE وظهرت اختلافات بين حزم البروتين باختلاف التركيب الوراثي. كما تم استخدام ثلاثة بادئات وراثية من RAPD وكانت البادئات الوراثية ناجحة في توليد حزم متضاعفة مع كل التراكيب الوراثية.