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EGYPTIAN ACADEMIC JOURNAL OF
BIOLOGICAL SCIENCES
BOTANY

ISSN 2090-3812

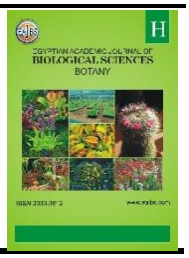
www.eajbs.com

Vol. 12 No.2 (2021)

Citation: Egypt. *Acad. J. Biolog. Sci.* (H. Botany) *Vol.12(2) pp41-57(2021)*
DOI: 10.21608/EAJBSH.2021.191436

Egypt. Acad. J. Biolog. Sci., 12(2):41- 57(2021)

Egyptian Academic Journal of Biological Sciences
H. Botany
ISSN 2090-3812
www.eajbsh.journals.ekb.eg.



Estimation of the Variation Coefficient and Some Local Genetical Resources of Some Common Bean

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ARTICLEINFO
Article History
Received:15/6/2021
Accepted:17/8/2021

***Keywords*:**
Common bean, heritability, coefficient of variation, RAPD marker

ABSTRACT

The present investigation was carried out during two successive summer seasons of years 2019 and 2020 at the Faculty of Agriculture (Saba Basha), Alexandria University and the laboratory of the vegetable seeds of Sabahya Horticulture Research Station, Alexandria Governorate, Egypt to evaluate six local cultivars and landraces of common bean for some morphological characters, yield and its components as well as estimate some genetic parameters and analysis. Results reflected obvious differences among the six genotypes of common bean for most of the studied characters. In general, the coefficient of variation was low (less than 10 %) or relatively low (less than 20 %) for most of the studied traits in the majority of studied genotypes of common bean. The highest coefficient of variation was obtained by Alexandria landrace (35.7 %) followed by Dandara landrace (27.9 %) in height of the first pod. These results indicated that the six genotypes of the common bean are genetically identical concerning all the studied traits except for the height of the first pod with respect to Alexandria and Dandara landraces. Analysis of variance data refers to that there were highly variations between genotypes under study. So, it can be concluded that all studied traits could be improved through the selection method. The number of days to the first pod is strongly affected by the change in the environmental conditions. Genotypes contain a fair amount of variations, and thus starting a breeding program consisting of self-reproduction and selection may be very effective in promoting productivity across different generations. All genotypes under study are considered fertile environment for breed selection and breeding, especially Assiut and Kafr El-shikh genotypes because its high productivity and good differences but it needs some improvement. Cluster analysis, according to DNA- RAPD analysis and morphological traits divided the 6 studied genotypes into groups. Among these clusters, there was a mono-genotypic cluster and the other included between 2 to 5 genotypes with a number of sub-clusters. The two methods assessed a high level of genetic variations. Based on results for morphological and molecular genetic diversity estimates, mono-genotypic clusters can be exploited to harness their unique features in breeding programs.

INTRODUCTION

Common bean (*Phaseolus vulgaris* L.) revels to the Fabaceae family, is an outstanding pulse crop with more than 35 million ha cultivated / year worldwide and is a globally important source of dietary protein to millions of people (Broughton *et al.,* 2003). The main categories of common beans, on the basis of use, are dry beans (seeds harvested at complete maturity), snap beans (tender pods with reduced fiber harvested before the seed development phase) and shell (shelled) beans (seeds harvested at physiological maturity) (Fahad *et al.,* (2014). Its leaf is also occasionally used as a vegetable and the straw as fodder. Common bean is a staple food in several countries and is found in local recipes that use fresh or dried grains. Common beans are healthy food due to high concentrations of several minerals and low concentrations of toxic elements in the grains (Di Bella *et al.,* 2016).

Characterization of crop germplasm using genetic markers provides estimates of genetic diversity, information which is essential for rational utilization of genetic resources in breeding programs. Conventionally, plant genetic diversity is estimated using variations in morpho-agronomics traits such as yield, resistance, tolerance, color and size (Szilagyi *et al.,* 2011). The genetic variability present in the bean (Phaseolus vulgaris L.) germplasm that is currently used as an agricultural crop has been shown to be stable in production and is acceptable for human sustenance. Accordingly, to maintain as much of the available variability as possible (Carias *et al.,* 2018). Breeding programs need to work with magnitudes of genetic variation in order to achieve the best results (Elshafei *et al.,*2019). Common bean is a source of dietary protein and the second most important legume crop in Africa next to faba bean, especially in Egypt. Hence, the development of commercial varieties is one of the major tasks to meet the increasing demand of the stakeholders. To this effect, understanding the genetic variability, heritability and association between grain yield and other agronomic traits is necessary for an effective plant breeding program (Yohannes *et al.,* 2020).

Analysis of genetic relationships in crop species is an important component of crop improvement programs, as it serves to provide information about genetic diversity, and is a platform for the stratified sampling of breeding populations. Traditionally, diversity is assessed by measuring variation in phenotypic traits such as flower color, growth habit, or quantitative agronomic traits like yield potential, stress tolerance, etc., which are of direct interest to users. This approach has certain limitations: genetic information provided by morphological characters is often limited and expression of quantitative traits is subjected to strong environmental influence. Different molecular markers have been used to study genetic diversity among common beans. The high-density linkage map of the common bean was developed using RAPD marker (Freyre *et al*., 1998).

RAPD is a PCR-based technique for identifying genetic variation. It involves the use of a single arbitrary primer in a PCR reaction, resulting in the amplification of many discrete DNA. RAPD technology provides a quick and efficient screen for DNA sequence-based polymorphism at a very large number of loci. The major advantage of RAPD includes that, it does not require pre-sequencing of DNA. The vast range of potential primers that can be used, gives the technique great diagnostic power. Reproducible RAPD bands can be found by careful selection of primers, optimization of PCR condition for target species and replication to ensure that only reproducible bands are scored. RAPD analysis has been extensively used for various purposes which include identification and classification of accessions, identification of breeds and genetic diversity analysis (Cao and Oard, 1997).

Due to the existence of a number of varieties of common bean grown on a commercial scale but not registered consequently, their exact characterizes are not known. So, this investigation was carried out for studying the morphological and genetic differences within

and between these unregistered varieties as a first step towards registering them, if they were genetically pure or including them in breeding programs to improve and establish new varieties. Thus, the current investigation was aimed to; 1) Performance evaluation and characterization of some common bean landraces in some qualitative and quantitative characters under open field conditions, 2) Estimation of some genetic parameter i.e., variance components, heritability in a broad sense, genotypic and phenotypic coefficient of variance (GCV, PCV), and 3) Evaluate the efficiency in molecular analysis using RAPD markers based on PCR technique.

MATERIALS AND METHODS

The current investigation was implemented during the summer seasons of 2019 and 2020 under field conditions at Faculty of Agriculture (Saba Basha), Alexandria University and the laboratory of the vegetable seeds of Sabahya Horticulture Research Station, Alexandria Government Egypt to evaluate six local cultivars and landraces of common bean for some quantitative and morphological traits.

Plant Materials:

Plant materials for this study consisted of six genotypes of common bean (one local cultivar and six landraces). The sources of these genotypes are illustrated in Table (1).

Table 1. The studied common bean genotypes and their sources

Genotype	source
Dandara	Non-registered cultivar collected from Sohag Governorate
Alexandria	Landraces collected from Alexandria Governorate
Kafr El-Sheikh	Landraces collected from Kafr Al sheikh Governorate
Aswan	Landraces collected from Aswan Governorate
Assiut	Landraces collected from Assiut Governorate
Nebraska	Registered cultivar at Horticulture Research Institute

Field Evaluation:

The seeds of the 7 genotypes were sown on Feb 5th during 2019 and 2020 summer seasons. The six entries were, randomly, distributed on a randomized complete blocks design with 3 replicates under drip irrigation conditions. The seeds were sown in hills spaced 40 cm apart. Normal agricultural practices for common bean production, i.e., irrigation, fertilization, weeds and pest control were practiced as recommended.

Recorded Measurements:

The following measurements were recorded on individual plants in each entry.

Vegetative Measurements; i.e., Plant length (cm) Starting from the surface of the soil to the growing top) , Number of branches/plants

Fruiting Measurements; i.e., Height of the first pod (cm) Starting from the surface of the soil to the first pod appears), Number of days from sowing to the first pod appears (days)

Pod measurements: The following measurements were recorded on randomly 30 pods from each entry; Pod length (cm), Pod width (cm), Pod weight (cm), number of Seeds / pods.

Yield and Its Components; i.e., Number of pods / plants, Total pods yield / plant (g), Total seeds yield / plant (g), Number of seeds / pods.

Molecular Analysis:

Genomic DNA Isolation: Genomic DNA was extracted from the young leaves of the six common bean genotypes by using DNA extraction kits (Easy Pure Plant Genomic DNA Kit)

DNA samples were stored at -20◦C. DNA quality was checked by electrophoresis in a mini gel.

In the present study, RAPD marker was employed to evaluate the efficiency in diversity analysis of common bean genotypes. The sequences of the used primers are shown in Table 2. PCR reactions were performed in 20µl total volume, using 1µl from diluted DNA, 1µl of each primer for the amplification reaction, 10µl master mix (Taq Ready Mix PCR Kit from the fast gene) and 8µl ddH_2O (sterile water) for all reactions. The tubes were capped and placed in a thermocycler and the cycling was started immediately. Amplification protocol was carried out using PCR cycler 600 programmed for initial denaturation step at 94˚ C for 5 min, followed by 40 cycles each at 94˚C for 30 sec, annealing at 37◦C and extension at 72◦C for 1min.

Table 2: sequences of the RAPD primers used in the study.

Primers code	Sequence(5´-3´)
OPA2	*GTG ATC GCAG*
OPA07	*GAAAGGGGTG*
OP-B7	*CAG CAC CCA C*
Op-B1	*GTAGACCCGT*

The products of RAPD based PCR analyses were detected using agarose gel electrophoresis (1.5% in 1X TBE buffer) stained with ethidium bromide (0.3µl). PCR products were visualized on U.V. light; photographed and analyzed using Gel Analyzed soft wear program.

Statistical Procedures:

Data of the studied characters were, statistically, analyzed using a combined analysis of variance for the two evaluated seasons, according to Herbert *et al*. (1955) and as illustrated in Table (3). The differences among the various means were tested, using Duncan's multiple range tests. The program used in the analysis COSTAT version 3. 303, 2004.

Table 3: The combined analyses of variance

S.O.V.	D.F.	S.S.	M.S.	E.M.S.
Reps./y	y(r-1)	S.S.r/y	S.S.r/y/ y(r-1)	
Years (Y)	(y-1)	S.S.y	S.S.y/(y-1)	$\sigma^2_e + r\,\sigma^2_{gy} + gr\,\sigma^2_y$
Genotypes(G)	(g-1)	S.S.g	S.S.g/(g-1)	$\sigma^2_e + r\,\sigma^2_{gy} + ry\,\sigma^2_g$
G × Y	(y-1)(g-1)	S.S.gy	S.S.gy/(y-1) (g-1)	$\sigma^2_e + r\,\sigma^2_{gy}$
error	y(r-1)(g-1)	S.S.e/y	S.S.e/y/y(r-1)(g-1)	σ^2_e

Genotypic and phenotypic variances were computed from ANOVA by Snedecor and Cochran (1980).Table based on the expected mean sum of squares as follows:

$$\sigma^2_{ph} = \frac{M.S.y. - M.S.E.}{g}$$

$$\sigma^2_g = \frac{M.S.g. - M.S.E.}{b}$$

$$(\,Environmental\ varians\,) \qquad \sigma^2_e = \mathrm{M.S.E.}$$

$$(\,Phenotypic\ varians\,) VPH = \sqrt{\sigma^2_{ph} + \sigma^2_g + \sigma^2_e}$$

$$(\,Genotypic\ varians\,) \qquad VG = \sqrt{\sigma^2_g}$$

Where; σ^2_g, σ^2_y, σ^2_{gy} and σ^2_{ph} types of variances of genotypes, years, genotypes × years interaction and phenotypes, respectively.

Genotypic (σ^2g) and phenotypic (σ^2ph) of variation were computed according to (Burton 1952).

$$\text{Genotypic coefficient of variance } (GCV) = \frac{\sqrt{\sigma_g^2}}{\bar{x}} \times 100$$

$$\text{Phenotypic coefficient of variance } (PCV) = \frac{\sqrt{\sigma_{ph}^2}}{\bar{x}} \times 100$$

Where: $\bar{x}$ = General mean of the trait

Broad sense heritability values were estimated for all studied traits as the ratio of genotypic variance (σ^2g) to the phenotypic variance (σ^2ph) and were expressed in percentage (Hanson *et al*., 1956).

$$(\ Heritability\ in\ broad\ sense\ )\quad H_{bs}^2 = \frac{\sigma_g^2}{\sigma_{ph}^2} \times 100$$

For molecular data and cluster analysis, data were scored for computer analysis on the basis of the presence of the amplified products for each primer. If a product was present in a genotype, it was designated as "1", if absent, it was designated as "0", after excluding the unreproducible bands. Pair-wise comparisons of genotype, based on the presence or absence of unique and shared polymorphic products, were used to determine similarity coefficients, according to Jaccard (1908). DNA fragment size was estimated by comparison with a 1500-kbp DNA ladder Ready to use from Gene Direx. The similarity coefficients were then used to construct dendograms, using the Unweighted Pair Group Method with Arithmetic Averages (UPGMA) from Past program version 4.03.

RESULTS AND DISCUSSION

Pictures in Plate (1) and results in Table (4) exhibited obvious differences among the six genotypes of common bean for most of the studied characters. Generally, Alexandria landrace was the earliest one concerning fruiting measurements (29.7 cm height of the first pod and 35.0 for a number of days to the first pod). Meanwhile, Dandara landrace was the latest one (53.1 cm height of the first pod and 48.3 for a number of days to the first pod). On the other hand, Assiut landrace gave the highest mean values for vegetative measurements (plant length was 2.8 m and No. of branches was 6.8). Meanwhile, Nebraska Cv. had the shortest plant (1.3m) and the least No. of branches (5.3). Regarding yield and its components, Assiut landrace exhibited the highest mean values for No. of pods/plant (64.3), total pods yield/plant (811.7 g) and total seed yield/plant (324.7 g). while the highest No. of seeds/pod was obtained by Alexandria landrace and Nebraska Cv. (6.6 for both). Pod of Nebraska Cv. surpassed the other genotypes concerning pod measurements. It was 14.9 cm in length, 1.5 cm in width, 14.4 g fresh weight and 8.6 g dry weight. Whilst, a pod of Dandara landrace had the lowest main values for all pod measurements. In general, the coefficient of variation was low (less than 10 %) or relatively low (less than 20 %) for most of the studied traits in the majority of studied genotypes of common bean. The highest coefficient of variation was obtained by Alexandria landrace (35.7 %) followed by Dandara landrace (27.9 %) in height of the first pod. These results indicated that the six genotypes of the common bean are genetically identical concerning all the studied traits except for the height of the first pod with respect to Alexandria and Dandara landraces. In this regard,

Analysis of variance in Table (5) showed that there were highly significant differences between genotypes in all characters understudies. These results indicate that there is a good amount of differences between the genotypes under study, which confirms

the possibility of improving these traits through selection and the beginning of a promising breeding program for these strains, however, the amount of improvement expected will depend on the amount of variation in each line. Similar results were reported by Broughton *et al.*, (2003), Bagheri *et al.*, (2017), and Fatema *et al.*, (2019). They showed that significant and highly significant differences between genotypes mean that these genotypes have a high gain of selection and beginning breeding program by selfing and selection may be very effective generation after generation. Some of the promising strains detected in the divergence study considered a treasure for plant breeders which breeding program based on it. Also, there were significant differences between both years of the study, in fruiting measurements, this can be interpreted as this property being affected by the different environmental conditions in two years of the study. Similar results were found by Kouam *et al.*, (2018) and Ghimire *et al.* (2019). They reported that the fruiting measurements of the traits that are affected by the change in environmental conditions. Concerning interaction between genotypes × years, there were no significant differences between genotypes in all traits under study.

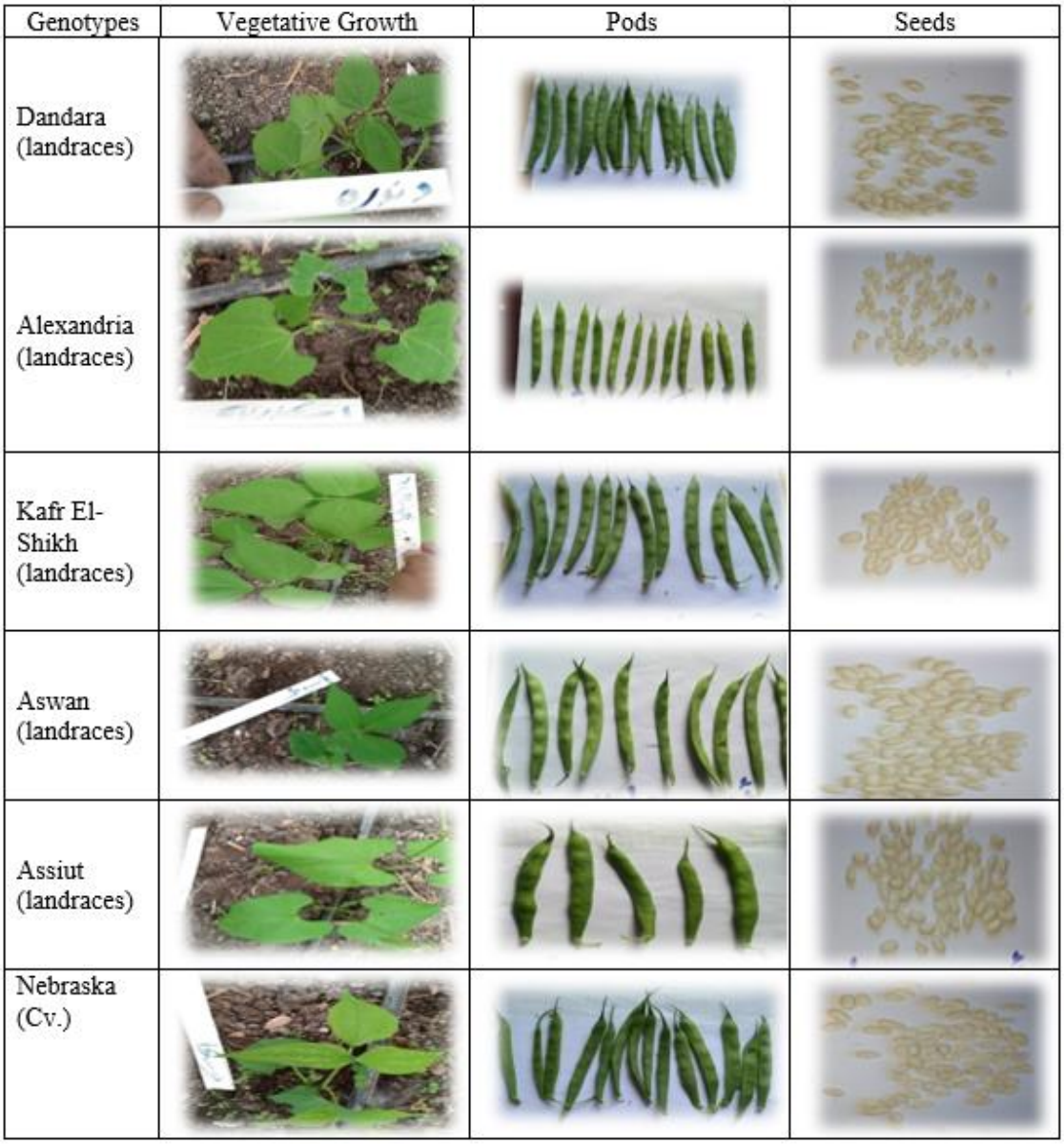

Plate 1. Pictures of the vegetative growth, pods and seeds of the six genotypes of common bean.

Table 4. Mean performance, range and coefficient of variation (C.V) of Fruiting, Vegetative and pod measurements, yield and its components of the six genotypes from common bean, calculated from the combined data over both 2019 and 2020 summer seasons.

Genotypes	Fruiting measurements						Vegetative measurements					
	Height of the first pod (cm)			Number of days to first pod			Plant height (m)			No. of branches / plant		
	X̄	R	C.V	X̄	R	C.V	X̄	R	C.V	X̄	R	C.V
Dandara (Lr.)	53.1 a	49.7 – 57.1	27.9	48.3 a	43.2 – 53.8	19.7	2.1 c	1.9 – 2.3	9.5	6.1 c	5.5 – 6.7	10.9
Alexandria (Lr.)	29.7 g	27.0 – 33.1	35.7	35.0 f	31.5 – 39.8	21.8	1.8 e	1.6 – 2.3	4.8	5.8 e	5.2 – 6.7	5.8
Kafr El-Sheikh (Lr.)	44.8 d	39.6 – 49.4	15.9	40.0 d	36.0 – 44.0	5.8	2.5 b	2.3 – 2.8	6.8	6.5 b	5.9 – 7.2	5.8
Aswan (Lr.)	49.6 b	45.0 – 53.0	6	44.2 b	39.6 – 48.3	5.8	1.9 d	1.7 – 2.1	10.2	5.9 d	5.3 – 6.5	11.9
Assiut (Lr.)	49.0 c	44.1 – 54.9	6.4	42.2 c	37.8 – 47.0	11.3	2.8 a	2.5 – 3.1	11.9	6.8 a	6.1 – 7.5	10.8
Nebraska (Cv.)	37.2 e	33.3 – 41.9	6	39.0 e	35.1 – 43.0	5.7	1.3 g	1.2 – 1.4	6.1	5.3 g	4.8 – 5.8	5.8
	Yield and its components											
	Number of pods / plants			Total pods yield / plant (g)			Total seeds yield / plant (g)			Number of seeds / pods		
	X̄	R	C.V	X̄	R	C.V	X̄	R	C.V	X̄	R	C.V
Dandara (Lr.)	52.5 c	37.7 - 67.3	18.2	515.8 f	477.0 – 550.3	5.4	206.3 f	190.8 – 222.5	5.4	4.4 e	4.1 – 4.6	5.3
Alexandria (Lr.)	43.9 e	39.6 - 46.3	11.9	589.8 d	534.6 – 643.7	5.6	236.0 d	213.8 – 256.5	5.6	6.6 a	5.9 – 7.2	7.8
Kafr El-Sheikh (Lr.)	65.0 a	58.5 - 72.5	6.1	783.8 b	702.0 – 863.7	15.9	313.5 b	280.8 – 346.5	15.8	5.7 c	5.1 – 6.3	5.5
Aswan (Lr.)	57.4 b	41.3 - 73.7	22.8	716.9 c	641.3 – 793.4	15.9	286.7 c	256.5 – 313.4	16.9	6.0 b	5.4 – 6.6	5.5
Assiut (Lr.)	64.3 a	57.6 - 71.7	6.2	811.7 a	720.0 – 892.1	25.8	324.7 a	288.0 – 353.7	20.9	6.1 b	4.4 – 7.8	16.8
Nebraska (Cv.)	39.4 f	35.1 - 44.2	5.8	566.0 e	509.0 – 616.5	5.8	226.4 e	203.6 – 249.0	5.8	6.6 a	6.0 – 7.2	15.3
	Pod measurements											
	Pod length (cm)			Pod width (cm)			Fresh pod weight (g)			Dry pod weight (g)		
	X̄	R	C.V	X̄	R	C.V	X̄	R	C.V	X̄	R	C.V
Dandara (Lr.)	10.3 f	8.5 – 12.3	15.4	1.0 f	0.8 – 1.2	15.8	9.8 f	9.0-10.6	5.4	5.9 f	5.4 - 6.4	5.4
Alexandria (Lr.)	14.0 b	12.6 – 16.2	7.2	1.4 b	1.3 – 1.5	5.6	13.5 b	12.2-14.6	7.3	8.1 b	7.3 – 8.9	7.3
Kafr El-Sheikh (Lr.)	12.6 d	11.3 – 13.5	5.5	1.3 d	1.1 – 1.5	14.4	12.1 d	10.8-15.0	5.6	7.2 d	6.5 – 7.9	5.5
Aswan (Lr.)	13.0 c	11.7 – 15.1	5.6	1.3 c	1.1 – 1.5	16.2	12.5 c	11.3-13.5	5.5	7.5 c	6.8 – 8.2	5.6
Assiut (Lr.)	13.1 c	9.7 – 16.3	16.2	1.3 c	1.2 – 1.4	5.5	12.6 c	11.3-13.8	14.8	7.6 c	6.8 – 8.4	12.8
Nebraska (Cv.)	14.9 a	13.5 – 15.4	7.4	1.5 a	1.4 – 1.6	5.4	14.4 a	13.1-15.8	17.4	8.6 a	7.8 – 9.4	16.3

Means with the same alphabetical letter in the column are not significantly different from each other using Duncan's Multiple Range Test at 5% probability.

Table 5. Mean squares of Fruiting, Vegetative and pod measurements, yield and its components for all genotypes under study, over two years of the study (2019 and 2020summer seasons).

S.O.V.	D. F.	Fruiting measurements		Vegetative measurements	
		Height of the first pod (cm)	Number of days to the first pod	Plant length (m)	No. of branches/plant
Blocks	2	0.03 ns	0.1 ns	0.0001 ns	0.0001 ns
Years(Y)	1	0.09 ns	0.49 *	0.00001 ns	0.00001 ns
Genotypes(G)	6	491.36 **	109.64 **	1.58 **	1.58 **
G x Y	6	0.15 ns	0.12 ns	0.0001 ns	0.00001 ns
Error	26	0.2	0.09	0.016	0.001
S.O.V.	**D. F.**	**Yield and its components**			
		Number of pods/plants	Total pods yield / plant (g)	Total seeds yield / plant (g)	Number of seeds / pods
Blocks	2	0.34 ns	80.26 ns	12.84 ns	0.002 ns
Years(Y)	1	0.11 ns	47.23 ns	7.56 ns	0.00009 ns
Genotypes(G)	6	577.71 **	95015.6 **	15202.5 **	4.21 **
G x Y	6	0.11 ns	55.86 ns	8.94 ns	0.02 ns
Error	26	0.95	142.81	22.85	0.02
S.O.V.	**D. F.**	**Pod measurements**			
		Pod length (cm)	Pod length (cm)	Pod length (cm)	Pod length (cm)
Blocks	2	0.004 ns	0.00004 ns	0.004 ns	0.001 ns
Years(Y)	1	0.00002 ns	0.000002 ns	0.00002 ns	0.00009 ns
Genotypes(G)	6	14.71 **	0.15 **	14.71**	5.3 **
G x Y	6	0.051 ns	0.00005 ns	0.05 ns	0.02 ns
Error	26	0.05	0.00005	0.05	0.018

** Highly significant differences at 1% level of probability.
Ns: not significant differences.

Variance components values in Table (6) show that the large portion of genotypic variance for the following characters: plant length, number of branches, pod length, pod width, fresh pod weight, dry pod weight, number of seeds / pod, number of pods / plant, total pods yield / plant, total seeds yield / plant and 100 seeds weight. Moderate values were in traits height of the first pod and number of days to first pod these results were in agreement with those found by Fahad *et al.,* (2014) and Yohannes *et al.,* (2020). They stated that the genotypic (GV) and phenotypic (PV) variability are considered the important criteria in a

successful breeding program and understand the genotypic difference of the most important quantity traits. It makes the breeding program by selection more effective and useful

Genotypic and phenotypic coefficient of variance values (GCV) and (PCV) showed in the same Table. The narrow range between the genotypic and phenotypic coefficient of variance was in all traits under study except for the height of the first pod and the number of days to the first pod, where the wider range was in it. These results were in harmony with those found by (Ejara *et al*., 2016). They stated that the traits which have a wider range between values of (GCV) and (PCV) meaning that these characters are more affected by the environmental conditions.

Heritability estimates broad sense showed in Table (6) show that differences between genotypic variance and phenotypic variance were narrow in the same traits which exhibited high heritability values. The highest heritability values were obtained, plant height, number of branches, pod length, pod width, fresh pod weight, dry pod weight, number of seeds / pods, number of pods / plants, total pods yield / plant, and total seeds yield / plant. Moderate values scored by traits height of the first pod and number of days to the first pod. Similar results were found by Maria and Mora (2008) and (Mammo *et al.*, 2019). They found that the highest heritability estimates scored in vegetative traits, yield components and pod measurements.

Table 6. Variance components values (σ^2G, σ^2E and σ^2PH) genotypic and phenotypic coefficient of variability (GCV, PCV) and heritability (over mean of 17 traits understudied in the common bean).

Traits	Variance					Coefficient of variability		Heritability in broad sense %
	σ^2Y	σ^2G	σ^2YG	σ^2E	σ^2PH	GCV	PCV	
Plant length	-0.00002	0.00404	-0.0003	0.001	0.005	0.19	0.23	84.47
Number of branches	-0.00003	0.005236	-0.0003	0.001	0.006	0.09	0.09	87.58
Height of the first pod	-0.003	0.38	-0.01	0.19	0.56	0.89	1.30	68.25
Number of days to first pod	0.02	0.13	0.01	0.091	0.25	0.31	0.59	51.61
Pod length	-0.00002	0.005	-0.00031	0.001	0.006	0.04	0.05	87.58
Pod width	-0.0002	0.002	0.0003	0.0004	0.002	0.15	0.18	79.74
Fresh pod weight	-0.002	0.21	0.000344	0.05	0.26	1.79	2.19	81.65
Dry pod weight	-0.0009	0.08	0.000124	0.02	0.09	1.07	1.32	81.65
Number of seeds / pod	-0.0009	0.14	0.000124	0.02	0.15	2.45	2.76	88.9
Number of pods / plant	-0.0002	7.68	-0.28	0.95	8.35	13.91	15.12	91.99
Total pods yield / plant	-0.41	1257.56	-28.98	142.81	1370.98	191.85	209.15	91.73
Total seeds yield / plant	-0.07	175.87	-4.64	22.85	194.02	67.08	73.99	90.65

σ^2_Y: Years variance, σ^2_G: Genotypic variance, σ^2_{YG}: Years ×Genotypes interaction, σ^2_E: Error variance, σ^2_{PH}: Phenotypic variance, PCV: Phenotypic coefficient of variance and GCV: Genotypic coefficient of variance.

Four primers for RAPD markers were screened for their ability to amplify the genomic DNA of the six studied common bean genotypes. Data were analyzed based on the comparison of the amplified fragments using gel documentation for each primer. If a fragment was present in a sample, it was designated as "1", if absent, it was designated as "0". If a fragment was present or absent in the genotype then absent or present in the others, it was called a unique species-specific marker, but if a fragment was absent and present in more than one genotype, it was called polymorphic finally if the fragments were present in all genotypes, it was called monomorphic.

A total of 100 RAPD fragments were amplified with the four used primers ranged from 17 (primer 2) to 38 (primer3), zero of them were common fragments (monomorphic), 16 of them showed to be polymorphic and other 83 showed to be unique fragments (Tables7 to 10 and plate 2).

Table 7. Amplified DNA fragments (AF) obtained for the six genotypes using first RAPD primers

Fragments	RF	MW	Genotypes						Polymorphism
			Nebraska	Assiut	Dandara	Kafr El-sheikh	Aswan	Alexandria	
1	0.306	950	0	0	0	0	1	0	Unique
2	0.36	766	0	0	0	0	1	0	Unique
3	0.391	675	0	0	0	1	0	0	Unique
4	0.395	665	1	0	0	0	0	0	Unique
5	0.407	634	0	0	0	0	0	1	Unique
6	0.422	595	0	1	0	0	0	0	Unique
7	0.426	585	0	0	1	0	0	0	Unique
8	0.434	567	0	0	0	0	1	0	Unique
9	0.469	490	0	0	0	1	0	0	Unique
10	0.484	458	1	0	0	0	0	0	Unique
11	0.504	422	0	0	0	0	1	0	Unique
12	0.516	401	0	0	0	1	0	0	Unique
13	0.523	388	1	0	0	0	0	0	Unique
14	0.535	368	0	0	0	0	1	1	Polymorphism
15	0.554	338	1	1	1	0	0	0	Polymorphism
16	0.589	288	0	0	0	1	0	0	Unique
17	0.605	268	0	0	0	0	1	0	Unique
18	0.609	263	1	0	0	0	0	0	Unique
19	0.694	171	0	0	1	0	0	0	Unique
20	0.721	147	0	0	0	0	1	0	Unique
21	0.729	141	0	0	0	1	0	0	Unique
Detectable fragments			5	2	3	5	7	2	

Table 8. Amplified DNA fragments (AF) obtained for the six genotypes using second RAPD primers:

Fragments	RF	MW	Genotypes						Polymorphism
			Nebraska	Assiut	Dandara	Kafr El-sheikh	Aswan	Alexandria	
1	0.316	534	0	0	1	1	0	0	polymorphic
2	0.319	528	0	0	0	0	1	1	polymorphic
3	0.358	465	0	0	0	1	0	0	Unique
4	0.361	460	0	0	1	0	0	0	Unique
5	0.365	455	0	0	0	0	1	0	Unique
6	0.368	450	0	0	0	0	0	1	Unique
7	0.400	408	0	0	0	0	1	1	polymorphic
8	0.460	342	0	0	1	1	1	0	polymorphic
9	0.463	339	0	1	0	0	0	1	polymorphic
10	0.467	335	1	0	0	0	0	0	Unique
11	0.537	278	1	1	0	0	0	0	polymorphic
12	0.544	273	0	0	1	0	0	0	Unique
13	0.547	270	0	0	0	1	0	0	Unique
14	0.554	266	0	0	0	0	1	1	polymorphic
15	0.579	250	0	0	0	0	1	0	Unique
16	0.582	248	0	0	0	0	0	1	Unique
17	0.618	229	1	1	0	0	0	0	polymorphic
Detectable fragments			3	3	4	4	6	6	

Table 9. Amplified DNA fragments (AF) obtained for the six genotypes using third RAPD primers

Fragments	RF	MW	Genotypes						Polymorphism
			Nebraska	Assiut	Dandara	Kafr El-sheikh	Aswan	Alexandria	
1	0.269	677	0	0	1	0	0	0	Unique
2	0.276	660	0	0	0	0	0	1	Unique
3	0.277	658	0	0	0	1	0	0	Unique
4	0.312	578	0	0	0	0	0	1	Unique
5	0.318	564	0	0	0	0	1	0	Unique
6	0.338	525	0	0	0	0	0	1	Unique
7	0.339	523	0	0	0	1	0	0	Unique
8	0.347	508	0	0	0	0	1	0	Unique
9	0.368	473	0	0	1	0	0	0	Unique
10	0.375	461	0	0	0	1	0	0	Unique
11	0.377	458	0	1	0	0	0	0	Unique
12	0.379	453	1	0	0	0	0	0	Unique
13	0.369	428	0	0	0	0	0	1	Unique
14	0.397	426	0	0	0	1	0	0	Unique
15	0.399	423	0	0	1	0	1	0	polymorphic
16	0.417	401	0	1	0	0	0	0	Unique
17	0.418	398	1	0	0	0	0	0	Unique
18	0.425	388	0	0	1	0	0	0	Unique
19	0.458	350	0	1	0	0	0	0	Unique
20	0.460	347	1	0	0	0	0	0	Unique
21	0.462	339	0	0	1	0	0	0	Unique
22	0.484	323	0	0	0	0	0	1	Unique
23	0.487	319	0	1	0	0	0	0	Unique
24	0.489	318	1	0	0	1	1	0	polymorphic
25	0.523	288	0	0	1	0	0	0	Unique
26	0.524	286	1	0	0	0	0	0	Unique
27	0.526	285	0	1	0	0	0	0	Unique
28	0.536	277	0	0	0	0	0	1	Unique
29	0.540	274	0	0	0	0	1	0	Unique
30	0.541	273	0	0	0	1	0	0	Unique
31	0.552	265	0	0	1	0	0	0	Unique
32	0.569	258	0	0	0	0	0	1	Unique
33	0.566	255	0	0	0	0	1	0	Unique
34	0.591	239	0	0	0	0	0	1	Unique
35	0.595	237	0	0	0	0	1	0	Unique
36	0.596	236	0	0	0	1	0	0	Unique
37	0.608	230	1	1	0	0	0	0	polymorphic
38	0.610	228	0	0	1	0	0	0	Unique
Detectable fragments			6	6	8	7	7	8	

Table 10. Amplified DNA fragments (AF) obtained for the six genotypes using forth RAPD primers:

Fragments	RF	MW	Genotypes						Polymorphism
			Nebraska	Assiut	Dandara	Kafr El-sheikh	Aswan	Alexandria	
1	0.266	622	0	0	0	0	0	1	Unique
2	0.309	540	0	1	0	0	0	0	Unique
3	0.319	521	1	0	0	0	0	0	Unique
4	0.326	510	0	0	0	1	1	0	polymorphic
5	0.369	445	0	1	0	0	0	0	Unique
6	0.397	408	1	0	0	0	0	0	Unique
7	0.404	399	0	0	0	1	1	0	polymorphic
8	0.408	395	0	0	1	0	0	0	Unique
9	0.411	391	0	0	0	0	0	1	Unique
10	0.440	360	0	0	0	0	1	0	Unique
11	0.443	356	0	0	0	1	0	0	Unique
12	0.450	349	0	0	1	0	0	0	Unique
13	0.461	339	0	1	0	0	0	0	Unique
14	0.479	322	1	0	0	0	0	0	Unique
15	0.496	307	0	1	0	0	0	0	Unique
16	0.504	301	0	0	1	0	0	0	Unique
17	0.511	296	0	0	0	1	0	0	Unique
18	0.518	290	1	0	0	0	0	0	Unique
19	0.553	266	1	0	0	1	0	0	polymorphic
20	0.557	263	0	0	1	0	0	0	Unique
21	0.564	259	0	0	0	0	1	0	Unique
22	0.567	257	0	1	0	0	0	0	Unique
23	0.624	225	0	1	0	0	0	0	Unique
Detectable fragments			5	6	4	5	4	2	

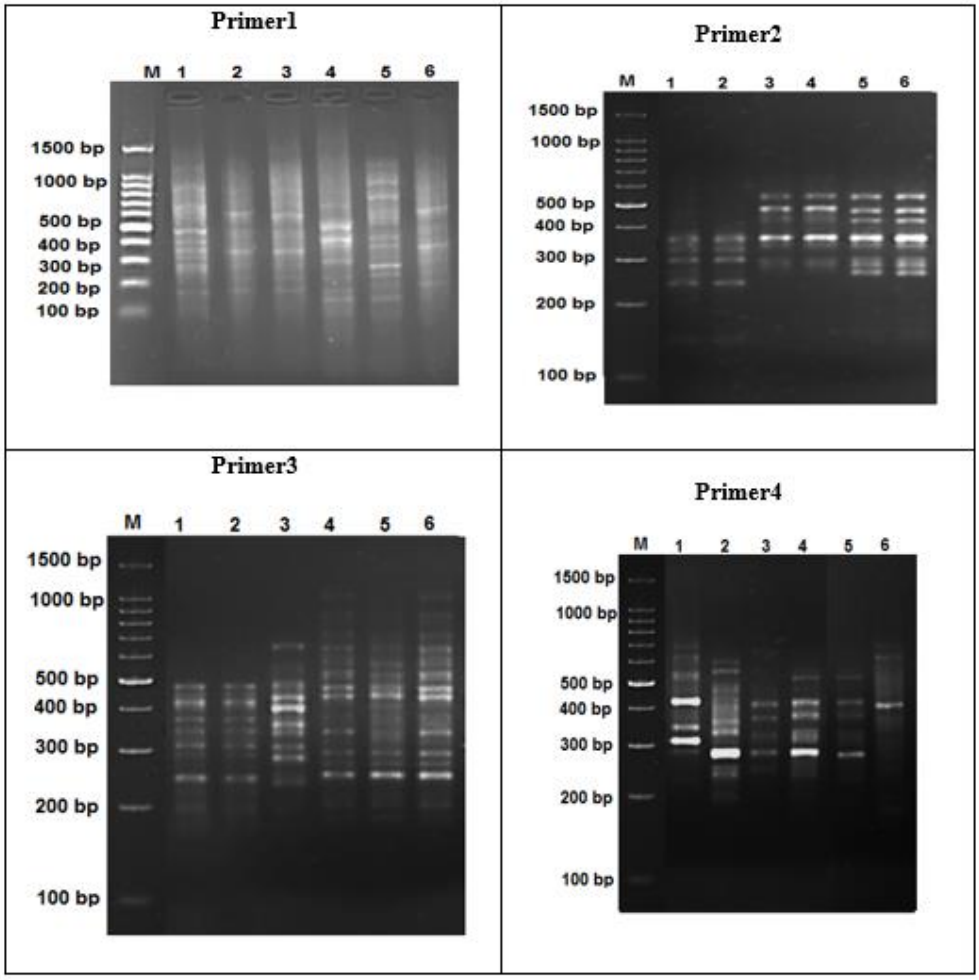

Plate 2: RAPD banding patterns in the six genotypes accessions generated using4 primers. (1, 2,3,4,5 and **6** for Nebraska, Assiut, Dandara, Kafr El-sheikh, Aswan and Alexandri, respectively).

Cluster analysis, according to DNA- RAPD analysis and morphological traits divided the 6 studied genotypes into groups as shown in Table (11) and Figure (1). Among these clusters, there was a mono-genotypic cluster and the other included between 2 to 5 genotypes with a number of sub-clusters. The two methods assessed a high level of genetic variations. Based on combined results for morphological and molecular genetic diversity estimates, mono-genotypic clusters can be exploited to harness their unique features in breeding programs.

Genotypes swapped among different clusters in different methods of clustering. Rahman et al. (2011) reported that genotypes also swapped from one cluster to another cluster among different methods and this pattern is somewhat irregular. These differences are not an indicator of the failure or limitation or weakness of the methods (Roldán-Ruiz, et. Al., 2001). These results may be due to the diversity at the molecular level, which may not reflect the diversity at the morphological or physiological level, as described by Karhu et al. (1996). Another possible reason for this variation in clustering might be the environmental influence and genotype-environment interaction. Compared to morphological and physiological characteristics, the DNA genome provides a direct comparison of genetic diversity at the DNA level, is phenotypically neutral and is not modified by environment and management practices (Messmer *et. al.*, 1993).

Table 11. Grouping of genotypes on the basis of morphological and molecular data by using PAST4.03 program:

P1	Genotypes	P2	genotypes	P3	Genotypes	P4	Genotypes	Four primers	Genotypes	Morphological	Genotypes
A	Nebraska ,Assiut and Dandara	A	Nebraska and Assiut	A	Dandra #	A	Assiut #	A	Nebraska #	A	Dandara#
A1	Nebraska#	B	Alexandria, Aswan, kafrelshik and Dandara	B	Nebraska ,Assiut, Aswan and kafrelshik	B	Dandara#	B	Assiut#	B	Aswan, kafrelshik, Assiut, Nebraska and Alexandria
A2	Dandra and Assiut	B1	Alexandria and Aswan	B1	Nebraska and Assiut	C	Nebraska , Aswan and kafrelshik	C	Alexandria, Aswan, kafrelshik and Dandara	B1	Aswan, kafrelshik and Assiut
B	kafrelshik	B2	kafrelshik and Dandara	B2	Aswan and kafrelshik	C1	Nebraska	C1	Alexandria and Aswan	B1a	Aswan#
C	Aswan and Alexandria			C	Alexandria#	C2	Aswan and kafrelshik	C2	kafrelshik and Dandara	B1b	kafrelshik and Assiut
						D	Alexandria#			B2	Nebraska and Alexandria

Mono-genotypic clusters

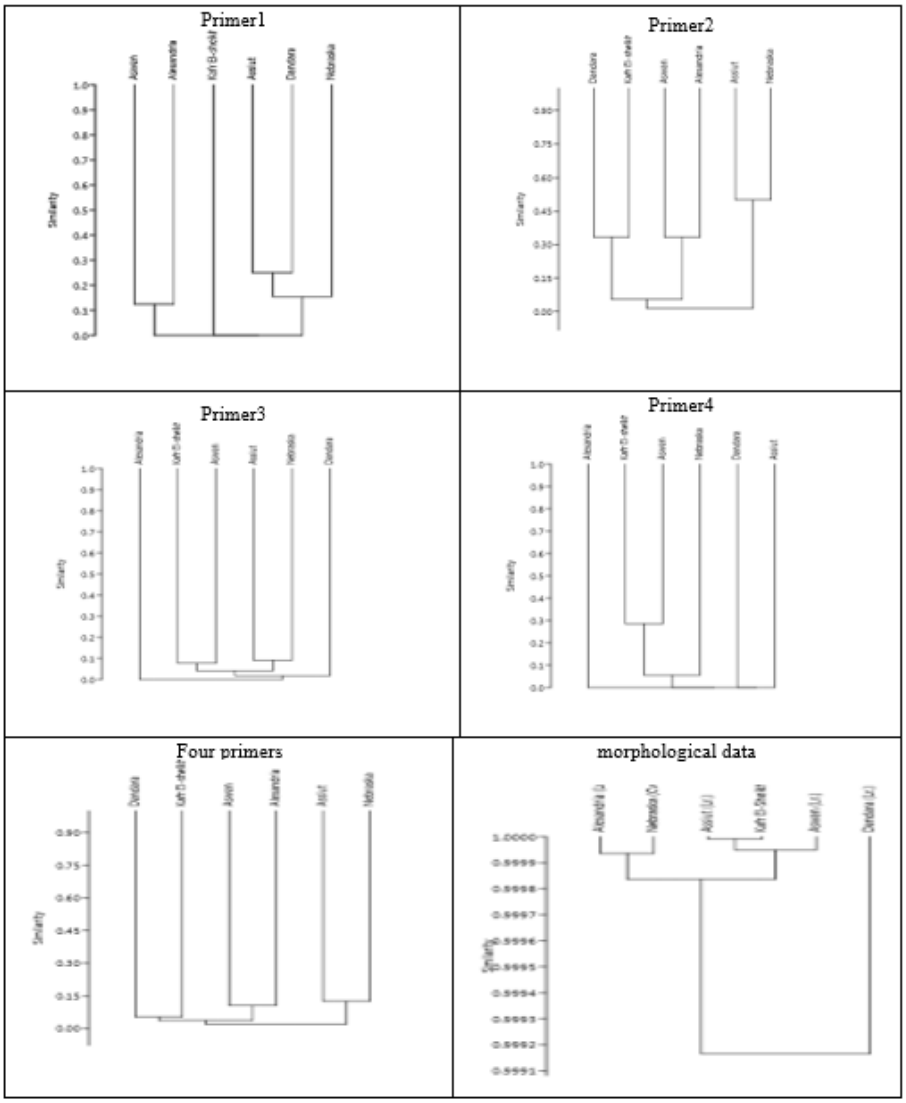

Fig.1: Cluster analysis using UPGMA method depicting genetic similarity (Jaccards coefficient) between six genotypes derived from band sharing data of RAPD and morphological data.

Diversity analysis of common bean germplasm resources in Slovenia and its vicinity, the Iberian Peninsula, and central Africa has confirmed the existence of gene introgression between the two gene pools (Blair et al., 2010). Gene introgression also exists in the common bean germplasm resources in China (Lei, 2018). According to the cluster analysis, there were many introgressed-type accessions (20%), suggesting that introgression between the two gene pools has occurred very frequently in Chongqing. This also suggests that the farmers in Chongqing have been selecting common bean germplasm resources for a long time to suit the agricultural conditions of the region.

It is essential to know the different ways that the data generated by molecular techniques can be analyzed before their application to diversity studies. Two main types of analysis are generally followed: (i) analysis of genetic relationships among samples and (ii) calculation of population genetics parameters (in particular diversity and its partitioning at different levels). The analysis of genetic relationships among samples starts with the construction of a matrix, sample × sample pair-wise genetic distance (or similarities). The advent and explorations of molecular genetics led to a better definition of Euclidean distance to mean a quantitative measure of the genetic difference calculated between individuals, populations, or species at DNA sequence level or allele frequency level. Genetic distance and/or similarity between two genotypes, populations, or individuals may be calculated by various statistical measures depending on the data set.

The percentage of polymorphic and unique bands obtained for the six genotypes are shown in Table (12). Genetic polymorphisms determine the diversity of individuals. Meanwhile, the number of specific AF and Amplified fragments obtained for the six genotypes are exhibited in Tables (13 and 14). The RAPD polymorphic and unique banding pattern analysis has been successfully used for molecular characterization and detection of genetic variability of genotypes in various crop plants.

Table 12: RAPD pattern of the six genotypes using 4 primers:

RAPD Primer	MB	PB	UB	TAF	P%	U%
1	0	2	19	21	9.5	90.5
2	0	8	9	17	47.1	52.9
3	0	3	35	38	7.9	92.1
4	0	3	20	23	13	86.9
Total AF	0	16	83	100	16	83
Average	0	4	20.7	25	4	20.7

Table 13: Amplified specific DNA fragments (AF) obtained for six genotypes using RAPD primers:

Primers	Genotypes						Total
	Nebraska	Assiut	Dandara	Kafr El-sheikh	Aswan	Alexandria	
1	4	1	2	5	6	1	19
2	1	0	2	2	2	2	9
3	4	5	2	6	5	8	35
4	4	6	4	2	2	2	20
Total	13	12	14	15	15	13	83

Table 14: Amplified DNA fragments (AF) obtained for the six genotypes using RAPD primers:

Primers	Genotypes						Total
	Nebraska	Assiut	Dandara	Kafr El-sheikh	Aswan	Alexandria	
1	5	2	3	5	7	2	24
2	3	3	4	4	6	6	26
3	6	6	8	7	7	8	42
4	5	6	4	5	4	2	26
Total	19	17	19	21	24	18	118

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ARABIC SUMMARY

تقدير معامل الاختلاف وبعض المقاييس الوراثية لبعض المصادر الوراثيه البلديه من الفاصوليا

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أجريت التجارب خلال الموسمين الصيفيين لعامي 2019 و 2020 بكلية الزراعة - سابا باشا، جامعة الإسكندرية ومعمل تقاوي الخضر بمحطة بحوث البساتين بالصبحيه بالإسكندرية. تهدف الدراسة الي تقييم ست سلالات محليه من الفاصوليا تم جمعها من مناطق الزراعة المختلفة بجمهورية مصر العربية بالإضافة إلى تقدير بعض المقاييس والتحليلات الوراثية.

النتائج التي تم الحصول عليها يمكن تلخيصها في التالي:

وقد أظهرت النتائج اختلافات واضحة بين التراكيب الوراثية الستة للفاصوليا في معظم الصفات المدروسة. شكل عام، كان معامل الاختلاف منخفضًا (أقل من 10٪) أو منخفضًا نسبيًا (أقل من 20٪) لمعظم الصفات المدروسة في غالبية التراكيب الوراثية المدروسة من الفاصوليا. تم الحصول على أعلى معامل اختلاف من السلالة المحلية الإسكندرية (35.7٪) تليها السلالة المحلية دندرة (27.9٪) في صفة ارتفاع القرن الأول. دلت هذه النتائج على أن الطرز السبعة للفاصوليا متطابقة وراثيا فيما يتعلق بجميع الصفات المدروسة باستثناء ارتفاع القرن الأول فيما يتعلق بسلالات الإسكندرية ودندرة.

يشير تحليل بيانات التباين إلى وجود اختلافات كبيرة بين التراكيب الوراثية. لذلك يمكن استنتاج أنه يمكن تحسين جميع الصفات المدروسة من خلال طريقة الانتخاب. تأثرت صفة عدد الأيام حتى ظهور القرن الاول بشدة بالتغير في الظروف البيئية. تحتوي الطرز الوراثية على قدر لا بأس به من الاختلافات ، وبالتالي فإن بدء برنامج تربية يتكون من التلقيح الذاتي والانتخاب للسلالات المتميزة قد يكون فعالاً للغاية في تعزيز الإنتاجية عبر الأجيال المختلفة. تعتبر جميع الطرز قيد الدراسة بيئة خصبة لانتخاب وتربية السلالات وخاصة طرز أسيوط وكفر الشيخ بسبب إنتاجيتها العالية واختلافها الجيد ولكنها تحتاج إلى بعض التحسين. تتناقص الاختلافات بين قيم الصفات المختلفة جيلًا بعد جيل حتى تصل العشيرة قيد الدراسة إلى قدر كبير من التشابه بين السلالات.

قسم التحليل العنقودي، وفقًا لتحليل DNA-RAPD والصفات المورفولوجية ، الأنماط الجينية الستة المدروسة إلى مجموعات. من بين هذه المجموعات كانت هناك مجموعة أحادية النمط الجيني والأخرى تضمنت ما بين 2 إلى 5 أنماط وراثية مع عدد من المجموعات الفرعية. أعطت الطريقتان مستوى عال من الاختلافات الجينية. وبناءً على النتائج المجمعة لتقديرات التنوع الجيني المورفولوجي والجزيئي ، يمكن استغلال العناقيد أحادية النمط الجيني لتسخير ميزاتها الفريدة في برامج التربية.