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Journal of Global Agriculture and Ecology

13(4): 1-16, 2022
ISSN: 2454-4205

GENETIC DIVERSITY AND PRINCIPLE COMPONENT ANALYSIS (PCA) OF FABA BEAN LANDRACES BASED ON YIELD-TRAITS AND PROTEIN SDS-PAGE

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This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Received: 10 January 2022
Accepted: 16 March 2022
Published: 19 March 2022

Original Research Article

ABSTRACT

This investigation was carried out at Bahteem farm Genetic resources research department ,field crop research institute , Agricultural Research Center ,Giza , Egypt during two growing seasons 2018/19 and 2019/20. Twenty landraces with two faba bean check landraces were grown in Randomized Complete Block Design (RCBD) with 3 replications to determine the genetic variability, morphological diversity and relationships among these landraces for agro-morphological and biochemical characteristics. Analysis of variance revealed high variability among all measured genotypes with respect to all agronomic and protein characteristic studied traits. Principle Component Analysis (PCA) was performed; the first principal component had 54.40% and 37.90% of the total variation (PC1) and the second principle component (PC2) explained 14.30% and 24.90% of the total variation for morphological and biochemical traits, respectively . The cumulative ratio of the first six primary components explained all variations of total variation. The studied faba bean landraces were distribution among PC biplot and clustered indicated distribution of studied material (matched with measured checks) by cluster or heat-map analysis. The results clustered or distributed differently based on morphological and biochemical traits. Then, GT biplot used to clear the relationship among the studied faba bean traits and landraces, showing that number of seeds and pods were the most positive effective traits in faba bean seed yield, causing highest harvest index. Results revealed that landrace G16 and G19 with the highest check Giza716 recorded the highest values of seed yield, number of seeds, number of pods and harvest index. GT biplot graph is a good preferred alternative procedure for each of correlation and cluster analyses and considered an effective technique beside or instead of cluster analysis for facility the interpretations. From all results, this work has provided useful data for elaboration of strategies for the conservation and sustainable management of the better genetic source germplasm and for *Vicia faba* improvement genetically.

Keywords: *Heat-map; cluster; local landraces; Principle Component Analysis (PCA).*

1. INTROUCTION

Faba bean (*Vicia faba* L.) is the most important legume in the world [1], especially in Egypt. Faba bean is a grain legume and grown for its high protein content (25.4%) in the seed [2]. The green immature beans are boiled and eaten as vegetable the mature seeds can be used for feeding livestock, swine and

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equine and poultry animals, and also play very important role in sustaining soil fertility by adding atmospheric nitrogen and organic matter to the soil. Cultivation declining is continuously due to the low yield and yield instability [3,4] with many problems that cause limits the production and reduce the growing area of this legume. All these constraints caused a negative trend in maintaining the numerous old cultivars present in different growing areas .This caused a strong genetic decline of the local gene pool with the real risk of losing useful genetic resources for future breeding programs and variety development (DeGior and polignano, 2001). The locally land races could represent as an economically valuable opportunity for farmers in marginal areas and become the basis for plant breeder to development varieties [5,6,7].

Yield trait is a complex component and created by many of morphological and physiological traits that correlated each other. Plant height, number of branches and pods per plant, biological yield, harvest index, 100-seed weight, days to flowering and maturity are the most important components in faba bean improvement for increasing seed yield [8,9,10].

Statically, techniques including principal components analysis (PCA) and biplot analysis have been successfully used to classify and measure the pattern of genetic characterization [11]. Correlation analysis was used widely in many crops by plant breeders to define the nature of complex interrelationships between yield components and to identify the sources of variation in yield knowledge derived in this way can be used to develop selection criteria to improve grain yield in relation to agricultural practices [12,13]. Principal components analysis (PCA) is a widely used tool in analyzing genetic diversity between plant landraces and determining the most important variables contributing to variation [14]. Biplot model is established by plotting the first two principal components (PC1 and PC2); however, it can also be equally used for all types of two-way data that assume a two way structure.

Protein banding pattern was efficiently used to identify landraces. Some protein markers were found to be related to yield traits that are very valuable in crop breeding. Some scientists used the protein by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) to assess the genetic diversity of some *Vicia* species [15,16] Abdel-Razzak et al. [17] and AlSaady et al [18].

The overall results indicated that SDS-PAGE was a useful tool for genetic diversity analysis and laid a solid foundation for future bean breeding. Adhikari Kedar [19] studied conventional breeding and molecular markers. Moreover, Warsame et al., [20] found preliminary investigations into the inheritance of traits. Protein data indicated the potential for genetic improvement, but not much has been achieved so far, due in part to a lack of genetic information associated with the complex relationship between protein content and grain yield, as this review indicates to current knowledge. The structure, composition and genetic control of *V. faba* seed storage proteins, highlighting key areas for further improvement of their content and composition based on recent advances in faba bean genetic tools. The cluster analysis also distributed the 101 landraces into six main groups, which was parallel to the systematic classification of Egyptian faba bean performed in previous studies Hou et al., [21]. Furthermore, Warsame et al., [22] screened 35 diverse *V. faba* landraces by using the one-dimensional dodecyl sulfate-polyacrylamide gel electrophoresis method (1D SDS-PAGE), and 35major protein bands obtained from three landraces with contrasting seed protein profiles were analyzed. In addition to, Qahtan et al., [23] explored genetic diversity in *V. faba* worldwide collection.

Due to the agronomic and economic interest these genotypes represents, the objective was to determine the genetic variability and relationship between faba bean populations by yield traits and protein markers, in views of identifying and selecting genetically contrasting accessions. Results will allow using the detected genetic variability and improvement of the production and also at a scientific level they serve as reference as stable faba bean material in breeding programs under different environments. Genetic diversity and the relation between faba bean accessions using physical and biochemical markers detected variability related to the gathering sites and found well-defined groupings of accessions according to the geographic region these accessions came from [12,24,18]. local populations from the Mediterranean recorded high variation levels in them and at least two genetically different groups.

GGE (landrace and genotype-by-environment) biplot model is established by plotting the first two principal components (PC1 and PC2); however, it can also be equally used for all types of two-way data that assume a two way structure. The landraces can be generalized as rows and the multiple traits as columns. Yan et al. 2001 used a landrace by trait (GT) biplot, which is an application of the GGE biplot technique to study the landrace by trait data.

The objective of this investigation was to : 1) examine genetic diversity and relationship among 20 faba bean

accessions collected from different regions in Egypt and two commercial cultivars, 2) use morphological traits and protein character as a marker assisted selection to high yield and protein to help in improvement the selection process, as well as 3) identify the most convenient landrace and trait interaction of faba bean using GT biplot and heat-map techniques to use the desired selected germplasms for the improvement of the production and also at a scientific level they serve as reference material in faba bean breeding programs.

2. MATERIALS AND METHODS

2.1. Plant Materials and Field Layout

The twenty faba bean (*V. faba* L.) landraces were obtained from different agro-climatic zones and market centers of Egypt, and two check varieties (Misr 1 and Giza 716) as shown in Table 1). All the twenty two landraces were sown over two seasons (2018-2019 and 2019-2020) at farm of Bahteem Research Station, Genetic resources research department ,field crop research institute , Agricultural Research Center ,Giza , Egypt (30° 28″ N, 31° 11″ E),). These experiments were laid out in a Randomized Complete Block Design (RCBD) with three replications. The seed of faba bean landraces were cultivated on the 15th of November for the first season and on the 20th of November for the second season .They were then planted on four ridge plots; 3m in length with 60 cm distances between the ridge and 20cm a part. Standard growing practices for the region were employed as recommended and plots were immediately irrigated after sowing as recommended.

2.2 Phenotypic Traits

At maturity , Five guarded plants were used to measure the agro-morphological traits ; plant height was measured from the soil surface to the upper most tip of the plant (PH) , first node length (FNL), the number of pods per plant (NP/P) , the number of seeds per plant (NS/P), seed yield per plant (SY/P), seed index (SI), days to 50% flowering (DF), biological yield per plant (B/P). Harvest index (%) = Seed yield plant-1/ Biological yield plant-1 × 100.

2.3 Extraction of Water-soluble Proteins

The samples were run at 60 V until entered the separation gel, then the voltage was raised to 110 V. Molecular mass protein standards from 20 to 250 kDa; PageRuler Broad Rang Unstained Protein Ladder, #26630, Thermo Scientific. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was used to study the banding patterns of the studied landraces according to Laemmli, [25] as modified by Studier [26].

Table 1. Code, Origin and obtained location of the studied 22 faba bean landraces

No	Code	Origin	location
G1	1	Aneba village by Wadi harit	Aswan
G2	2	3km of E of Aneba along Wadi kharit	Aswan
G3	17	5km s of Kom-Ombo on the main road to Aswan	Aswan
G4	26	6km SE of Kom-Ombo on the road to Waid Kharit	Aswan
G5	29	13km SE of Kom-Ombo on the road to Waid Kharit	Aswan
G6	53	38Km N of Kalabsha, by the main road	Aswan
G7	64	4km N of Isna by the left bank road	Qena
G8	66	Kiman EL- Matana	Qena
G9	107	18km S of Armant	Qena
G10	334	11km N of Tahta	Sohag
G11	341	9km S of Sidfa	Sohag
G12	403	14km W of Assiut	Assiut
G13	405	25km N of Assiut	Assiut
G14	451	12km W of EL-Minia	Minia
G15	485	3km S of Beni Mazar	Minia
G16	512	11km S of Ihnasya EL madina	Beni suef
G17	638	3km of EL-FAYOUM (EL Karasdisa	Fayoum
G18	784	5km E of Zagazig	Sharkia
G19	825	5km W of Mit-Ghamr	Dakahlia
G20	830	5km E of Tanta	Gharbia
G21	Misr 1	Giza 3x123 a/45/76	Egypt
G22	Giza 716	461/842/83x503/453/83	Egypt

2.4 Statistical Analysis

The data of each season was forwarded to analysis of variance (ANOVA). Before running the combined analysis, Levene test [27] was applied to study the assumption of variances homogeneity. A combined analysis of variance of randomized complete block design over the two growing seasons was performed using GenStat computer software as outlined by Snedecor and Cochran [28]. Least Significant Difference (LSD) test for means was used to detect the significant differences among genotype.

Simple correlation coefficients between all pairs of the studied traits were computed as suggested by Snedecor and Cochran [28] and [29]. The principal component (PC) analysis was applied to assess the herein collected data. The first two PCs were used to group the landraces, whose values were used to generate the biplots; PC1 was used on the horizontal axis, whereas PC2 was used on the vertical axis as described by [30]. Hierarchical cluster analysis was performing on the standardized data using a measure of Euclidean distance and Ward minimum variance method as outlined by Ward [31].

The GT biplot method [32] was used to show the faba bean by trait two-way data in a biplot. These statistical methods have been described in detail by Loss et al. [33], Yan et al [32] and Yan and Rajcan [30]. All biplots presented in this study were generated using the GenStat package [34].

Gels were visualized and scored with Alphaimager 2200 software Version 4.0.1. All scored microsatellite data employing the Arlequin 3.11 software package after data conversion using CONVERT program and POPGENE software package [35]. Dendrogram was built using un-weighted pair-group method with arithmetical average (UPGMA) cluster analysis based on Jaccard's similarity coefficient using PAST Software (PAleontological Statistics Version 1.94b). Protein polymorphic bands registered on 12% gel of separating poly-acrylamide gel wells and SDS-PAGE were processed in R software to produce the module of heat-map.

3. RESULTS AND DISCUSSION

Results of Levene test [27] confirmed that all studied traits had the homogeneity of variances for that permits applying the combined analysis. Accordingly, the combined mean performance of the evaluated landraces over the two seasons for the yield traits is presented in Table (3). Combined analysis of variance across two seasons cleared that years were significant for all traits except for flowering date. These results indicated that environment had significant effect on the performance of studied faba bean landraces. However, landrace x year interaction was insignificant for all studied traits except for pod length, number of seed/plant and seed yield /plant. This indicates the presence of sufficient variability. These results are in conformity with the results reported by [36].

3.1 Morphological Faba Bean Traits

3.1.1 Mean performance

The combined mean performance revealed significant differences among landraces for all nine studied traits in separate analysis and combined ones (Tables 3). The considerable variability among the landraces provides a good chance to improve the faba bean yield traits.

Data showed that Landrace (G13) recorded the tallest plants (110.90 cm) and G22 was the shortest one (91.03 cm) with. Landrace (G5) possessed the lowest length of the 1st node (13.33 cm) while, landrace (G22) was the highest one (22.43 cm). Meanwhile, G22 (Giza716) recorded the highest values for pod length (10.10 cm).

Whenever, landrace (G16) produced highest number of pods/plant (17.45 pods) with the highest number of seeds/plant (51.47 seed) producing, the heaviest weight of seed yield/plant (47.88 g per plant) and biological yield (111.72 g) recording, with the highest harvest index (44.34). Landrace (G14) was the earliest landrace recording 51.85 days while, landrace (G22) was the latest one 62.48 days.

Generally, results showed that landraces 16 and 19 surpassed the highest check landrace (Giza716) and other tested landraces for seed yield/plant by increasing ratio 9.74 and 7.43, respectively over the best check genotype. Landraces are considered one of the sources of variation in breeding programs. They are an important factor in raising faba beans, as they help improve beans, which is an important factor as the sources of variation are landraces, imports and old varieties. Here these breeds collected from the farmers help in adding traits that are not in the base of variations. Similar results were reported by several investigators such as and Abdel-Razzak et al. [17], Ghareeb and El-Emam [37], Abdalla et al. [38] and AlSaady et al. [18].

3.2 Relationships among Phenotypic Studied Traits

Many statistical analyses were applied to study relationships between phenotypic studied traits as simple correlation coefficient and the principal component analysis (PCA) analysis.

3.2.1 Correlation

All characters as a correlation matrix using excel sheets (Pearson 1895).Simple correlation coefficients among seed yield/plant and the other related traits are shown in Table (4). Results revealed that all traits were highly significantly association with seed yield/plant except for plant height (PH), first node height (FNL) and pod length (PL). The first flower showed highly negative significant relationship with seed number (-0.904**) its reverse correlation, pod number (-0.898**), harvest index (-0.750**) and biological yield (-0.532*). Meanwhile, positive significance value was obtained between number of pods/plant trait and seed number (0.960**), harvest index (0.735**) and biological yield (0.609*). However, number of seeds/plant was highly positive significant with biological (0.666**) and significant positive harvest index (0.779**).

Results showed that the most important positive associations to faba bean breeder were those between seed yield/plant (SY) and each of number of seeds/plant (0.964**), significant positive number of pods/plant (0.898**), significant positive harvest index (0.852**) and biological yield (0.614**). It is evident that the selection for the all or some of the previous traits would be effective in improvement the productivity of faba bean because of their positive and significant association with yield. However, days to 1st flowering (Flo) was negatively significant (-0.856**) with seed yield/plant, meaning that the early plant flowering can produces highest pod (Pod) and seed (Seed) number with heaviest biological (Bio-Y) and seed yields (SY), recording maximum harvest index (HI). This results agree with Alan and Geren [39], Haridy and El-Said [40], Ahmed et al., [41], Afzal et al., [42].

Table 2. Mean performance of the 22 faba bean landraces as a combination of the two seasons

Entry	PH	FNL	PL	Pod	SY	Flo	Seed	Bio-Y	HI
G1	104.58	21.97	8.10	11.43	27.57	62.48	33.62	94.57	29.15
G2	101.65	14.25	8.12	13.50	35.02	54.83	40.85	100.38	34.88
G3	106.62	16.43	10.10	13.15	34.65	55.68	39.02	99.95	34.67
G4	100.62	18.98	8.53	15.70	36.35	53.78	43.52	96.20	37.79
G5	101.95	13.33	7.10	16.55	40.30	52.28	47.90	94.33	42.72
G6	104.05	19.68	8.22	16.55	39.42	52.72	47.80	107.25	36.75
G7	104.92	16.85	7.10	16.33	38.80	53.08	47.23	102.32	37.92
G8	108.83	20.08	8.10	15.35	36.28	54.55	42.60	95.82	37.87
G9	101.03	16.43	8.30	12.52	31.38	58.77	35.88	96.03	32.68
G10	100.25	14.95	7.98	12.55	32.08	55.93	38.88	101.02	31.76
G11	103.65	19.13	8.43	15.27	35.72	54.60	42.50	99.37	35.94
G12	101.42	19.80	8.00	13.48	34.98	55.58	40.30	99.93	35.01
G13	110.90	22.02	7.87	15.75	36.48	53.57	44.68	104.22	35.01
G14	102.05	16.18	7.83	15.78	38.28	53.32	46.98	106.32	36.01
G15	106.70	20.33	7.72	16.78	42.83	52.28	50.60	102.48	41.80
G16	102.25	14.90	8.05	17.45	47.88	51.85	53.50	107.78	44.43
G17	94.47	20.02	8.08	12.80	34.28	55.77	38.90	82.65	41.48
G18	102.47	17.87	8.12	15.90	38.52	53.20	47.08	103.20	37.32
G19	107.52	18.28	7.73	17.33	46.87	52.25	51.47	107.87	43.45
G20	99.80	18.07	8.32	15.22	35.25	54.67	42.32	97.37	36.20
G21	102.90	19.98	8.58	15.77	38.15	53.48	45.85	107.32	35.55
G22	91.03	22.43	8.63	16.83	43.63	52.27	50.75	111.72	39.06
Mean	102.71	18.27	8.14	15.09	37.49	53.78	44.19	100.82	37.02
Homogeneity	1.98ns	1.03ns	1.08ns	1.54ns	1.28ns	1.04ns	1.32ns	1.13ns	1.30ns
LSD_ Year	1.06	1.16	0.48	1.35	4.68	NS	2.63	3.38	3.88
LSD_ Entry	5.64	2.19	0.48	2.51	5.87	2.39	4.12	5.18	6.34
Y*En	NS	NS	0.68	NS	8.30	NS	5.83	NS	NS

PH: Plant height, FNL: First node length, Pod: No. of pods /plant, PL: Pod length, SY: Seed yield /plant, Seed: No. of seeds/plant, Flo: Time of flowering, Bio-Y: Time of flowering and HI: Harvest index

Table 3. Correlation coefficients among faba bean seed yield components over 2018/19 and 2019/20 seasons (n=22)

	PH	FNL	PL	Pod	Flo	Seed	Bio-Y	HI	SY
PH	1								
FNL	0.026	1							
PL	-0.103	0.164	1						
Pod	0.145	0.020	-0.309	1					
Flo	-0.022	0.152	0.225	-0.898**	1				
Seed	0.052	-0.048	-0.317	0.960**	-0.904**	1			
Bio-Y	0.143	0.053	0.042	0.609**	-0.532*	0.666**	1		
HI	-0.076	-0.140	-0.321	0.735**	-0.750**	0.779**	0.113	1	
SY	0.024	-0.084	-0.232	0.898**	-0.856**	0.964**	0.614**	0.852**	1

PH: Plant height, FNL: First node length, Pod: No. of pods /plant, PL: Pod length, SY: Seed yield /plant, Seed: No. of seeds/plant, Flo: days to 1st flowering, Bio-Y: Biological yield and HI: Harvest index

The obvious results indicated that increasing number of pods/plant produced a higher number of seeds/plant that would be accompanied by seed yield/plant and high biological therefore increase the harvest index. Correlation coefficients between seed yield/plant and related traits not provide complete information about the importance of each component in the structure of seed yield, neglecting the complicated associations among all traits [43]. This is advantageous in that the landraces are adapted to climatic conditions, their management and their traditional uses in breeding programs. Although many of these landraces are considered immutable, they are in a state of constant evolution as a result of natural and artificial selection. The adaptation of the historical landraces also represents that they are compatible with the current local agricultural conditions within the region or the same country using the latest breeding techniques. Modern within a defined geographical area and under the influence of local human cultural practices. This also includes the adaptation of landraces to new management systems and selection made by growers or breeders using available technology. It is a mixed selection system in which farmers and other social actors develop advanced landraces .These results concur with those reported by those of Ibrahim and Ghareeb [44] and Abdalla et al. [38].

3.3 Principal Component Analysis

Principal component analysis (PCA) was carried out using the correlation matrix among studied traits for reduction data set of these traits by classifying the more correlated traits in one group. Results of principal component analysis was shown as tabulated in Table (5) and as graph by loadings of the first two principal components (PC1 and PC2) plotting (trait/genotype) against each other as biplot graph Fig. (1).

Results presented in Table (5) cleared that total variation was derived to 8 principal component and values of Eigen, Variability% and Cumulative%. The first two principal components (PC1 and PC2) had 54.40% and 14.30%, respectively from the total variation. The cumulative ratio of the two primary components in total variation was 68.70%. Meanwhile, the rest of other principle components recorded different proportion% (PC3=11.90%, PC4=9.70%, PC5=6.70%, PC6=2.10%, PC7=0.70% and PC7=0.10%) of the total variation. The all principle components explained 100% (as cumulative %) of the total variation. Then, all variations were exhibited in the first 7 principle components (PCs) in this study. These results were agreement with Yeken et al. [45] and Madakbas and Ergin [46] who explained all variations in the first 6 PCs. Eigen values of the first four PCs (PC1, PC2, PC3 and PC4) were above 1, indicating to reliable the evaluated principal component weight values [47] and was more informative than the original variable.

Graph illustrated in Fig. (1) showed the best visualization view for Principal component analysis. The first two primary components were graphically plotted using a simple form of biplot graph supported by Yan and Rajcan [30]. PC1 and PC2 loading were presented in the horizontal and vertical axes, respectively. Traits concluded narrower acute angle that located in the right side on higher quarter of graph were more correlated, while traits with obtuse angles have a negative correlation. A most important positive relationship that between seed yield/plant (SY) and number of pods (Pod) and seeds/plant (Seed), followed by harvest index (HI) and biological yield (Bio-Y) while was negative correlated with days to first flower. The current results are in harmony with correlation results and those obtained by Belal et al., [48].

Table 4. Principal component parameters Eigen values, proportion% of variance and cumulative values for studied faba bean traits

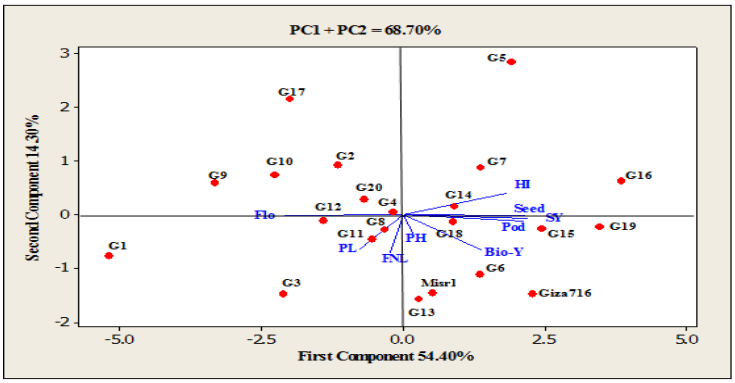
Parameter	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9
Eigen value	48.89	1.29	1.07	0.88	0.61	0.19	0.06	0.01	0.00
Proportion%	54.40	14.30	11.90	9.70	6.70	2.10	0.70	0.10	0.00
Cumulative%	54.40	68.70	80.60	90.40	97.10	99.20	99.90	100	100

The 20 Egyptians faba bean landraces with two checks were distributed among principal component analysis (PCA) biplot graph as illustrated in Fig. (1). These landraces had great diversity that was useful in breeding programs Belal et al., [48]. Results indicated that G16, G19, G15, Giza171, G5, G7, G6, G14, G18 and Misr1 gave the highest performance over the grand mean agree with Ibrahim and Ghareeb [44].

3.4 Cluster Analysis

Cluster analysis was used as effective tool for classified the 20 landraces with two checks into different classes as illustrated in Fig. (2). The graphically cluster analysis was shown in dendrogram presenting similarity among them. The 22 landraces had divided into three groups; first group included five Egyptian faba bean landraces with the highest heck (Giza716, G19, G16, G15 and G5) which they had highest seed yield (SY) and its performance of almost yield traits. The second group included eleven landraces (G13, G18, G7, G14, Misr1, G6, G8, G12, G11, G20 and G4). Meanwhile, third group concluded the last six landraces (G17, G9, G3, G10, G2 and G1).

Based on phenotypic traits, results of principal component and cluster analysis confirmed that the evaluated landraces (Giza716, G19, G16, G15 and G5) recorded the best performance for faba bean seed yield. Similar results were gained by Belal et al., [48].



PH: Plant height, FNL: First node length, Pod: No. of pods /plant, PL: Pod length, SY: Seed yield /plant, Seed: No. of seeds/plant, Flo: days to 1st flowering, Bio-Y: Biological yield and HI: Harvest index

Fig. 1. Principal Component (PC) biplot, showing the correlation relationships among the morphological traits and distribution of studied faba bean landraces

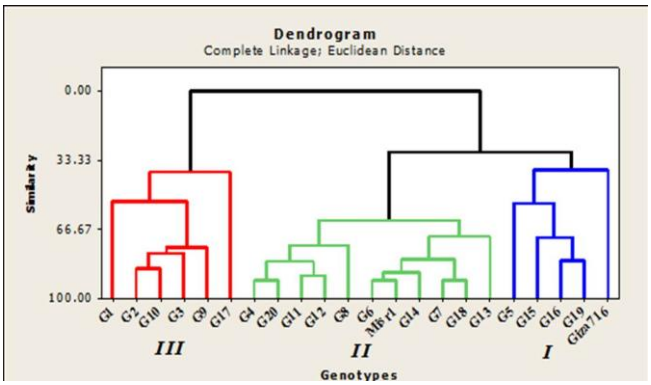


Fig. 2. Cluster dendrogram showing the distance among 22 faba bean landraces based on seed yield and its phenotypic related traits

3.5 Biochemical Hierarchical Cluster Analysis

SDS-PAGE of seed storage proteins (water-soluble fraction) was used to assess the genetic diversity of the studied 22 faba bean landraces. The electrophoretic banding patterns of proteins extracted from the seeds of the selected faba bean landraces were shown in Fig. (3). The presence and absence of bands were assessed with (1) and (0), respectively. The results of SDS-PAGE revealed a total number of 22 bands with molecular weights (MW) ranging from about 250 to 20 KDa, which were not necessarily present in all landraces.

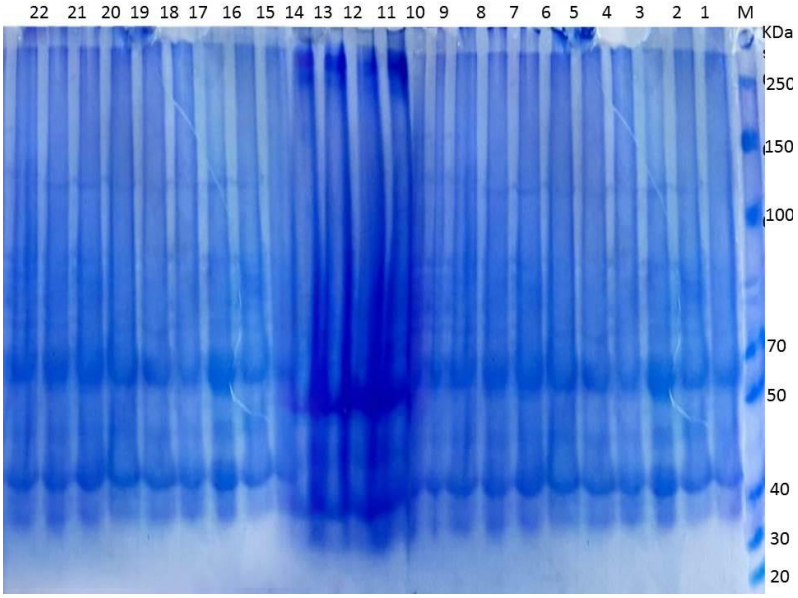
The results of the protein analysis showed 117 bands resulting from protein electrophoresis. The highest landraces producing fragments were No. 1 and No. 2 with seven fragments. The lowest yield of fragments was landrace No. 12 and No. 13 with 3 fragments.

The fragments were distributed as follows at 250 kDa. The number of fragments was 15 fragments that appeared out of 22 fragments, with a polymorphism of 68%. The lowest polymorphism was the appearance of a single and unique item in landrace 8 at a molecular weight of 150 kDa with a polymorphism of 5%. At 100 kDa there were 6 fragments with 27% polymorphism and at 70 kDa there were 64% polymorphism due to the presence of 14 fragments. At 50 kDa, there were 18 fragments with a phenotypic multiplicity of 82%. At 40 and 30 kDa, all bands appeared equal to 22 bands with a polymorphism of 100%. Polymorphism at 20 kDa, there are 19 bands, with a polymorphism rate of 86%. The overall percentage of polymorphism is 66%, and monomorphism represents 34%.

Dendogram and Heat-map

The biochemical traits (molecular bases) were used to construct two-way hierarchical cluster analysis using SDS-PAGE product to get relationship among studied faba bean landraces based multiple molecular bases. Fig. 4 illustrated multivariate heat-map illustrating the genetic diversity of 22 faba bean landraces, based on the SDS-PAGE for using the module of heat-map of R software.

The landraces were distributed in 3 main clusters. The first cluster contains landrace 12 and landrace 21 in one group, while landrace number 13 is in another group. I am the second cluster, and it includes landrace 10, 11. While the third cluster is located under two clusters, the first cluster contains three groups. The first cluster contains landraces 8, 16 and 18. As for the second group, it includes under a group that includes landraces No. 3 and No. 6, and under another group that includes landraces No. 22, 20, 14, 7, 5 and 4. And under the third group is located in which the landrace No. 9 alone.



M: indicate Molecular size marker. Numbers from 1 to 22: refer to the samples of the studied landraces name for faba bean

Fig. 3. Protein SDS-PAGE product profiles of twenty two investigated samples of faba bean landraces.

On the other hand, under the third cluster includes three groups, the first group includes landrace 15, and under the second group is landrace 1, 2, while the third group includes 17 and 19 as shown in Fig. 1. The same distributions are found in the heat map Fig. 3. As for the data in Table 1 it was announced that the highest percentage of similarity was 100% between landrace 1 and landrace 2. The lowest similarity percentage was 44% between landrace No. 12 and both landrace No. 17 and No. 19. The existence of different forms of distribution of different landraces does not mean that we are going to use all of them, but rather that these landraces carry a range of different differences, the most remote of which can be used in breeding programs as parents that can be crossed with other landraces or old varieties.

It is expected that local breeds have a certain half-life during which they are consistently maintained through conservative selection, while new, more sophisticated versions are being developed. Conservation taxa records represent an attempt to provide a framework for these materials, but further developments are needed to take into account the heterogeneity inherent in the original landraces.

3.5.1 Cluster tree of biochemical protein analysis

The biochemical trait (protein) was used to summarize hierarchical cluster tree analysis using SDS-PAGE product to get relationship among studied faba bean landraces. Fig. 5 illustrated cluster tree illustrating the genetic distance among 22 lines from Vicia faba based on the analysis of SDS-PAGE protein analysis using the Euclidean distance and the UPGMA algorithm in the PAST Software.

The landraces were distributed in 3 main clusters. The first cluster contained landraces G13, G21, G12 and G10 in one group, while landrace number G18, G16, G8, G6, G3, G22, G20, G14, G7, G5, G4 and G9 was concluded in another group. However, landraces G15, G17, G19, G2 and G1 formed independent cluster. Hou et al., [21] measured the genetic diversity and relationships between 101 faba beans (Vicia faba L.), the landraces and cultivars were analyzed by sodium dodecyl sulfate (SDS)-polyacrylamide electrophoresis (PAGE), and it was also found that the protein could be identified genetic diversity and measured by the protein. This means that there is divergence in the genotypes, and these genotypes can be used in breeding programs. Also, breeding programs can increase the differences by making crosses between genotypes with a long genetic distance to obtain other genotypes and different isolated generations that are promising varieties for farmers. Therefore, these genetic structures are considered a nucleus for breeding beans. These finding were agreed with those of Abdel-Razzak et al. [17].

3.5.2 Principle component analysis

Results of principal component analysis were shown as graph by loadings of the first two principal components (PC1 and PC2) for biotechnological trait Fig. (6). The landraces were divided into four axes. Firstly, right quarters included landraces as G8, G19, G16, G15 and G2 as the best ones. The left side quarters that contained the lowest landraces including another reset landraces as G13, G20, G12, G 9, G10, G11 and G6. These results were in agreements to those obtained by El-Sherif and Almutairi [49]. Studied Protein bands were induced in faba bean plants exposed to water deficit whereas marjoram extract caused a decrease in protein accumulation.

When the biplot graph of the landraces used in the study is examined (Fig. 6), it is seen that the landraces are quite different from each other and they are distributed in the graph. It is possible to say that only G19 and G16 landraces can be similar. This situation can be fully explained by the reflection of biochemical factor (protein) on the studied landraces. Similar results gained by Abdel-Razzak et al. [17] and Madakbas et al. [50]. As a result of the PCA analysis, 1st two principle component axes were obtained and these axes represented all of the total variation. Principal component analysis (PCA), which is a size reduction method using the data set of the studied agricultural characteristics, applied. The first principal component had 32.30% 37.90% of the total variation (PC1). The second principle component (PC2) explained 21.00% 24.90% of the total variation. Madakbas and Ergin [46] also reported that most variations were explained with the first two principle components.

3.6 GT-Biplot Graph on Phonotypical and Biochemical Traits

Under all the different previous results, analysis in this situation can be fully explained by the reflection of the all studied traits (morphological and biochemical characteristics). Combined data as similar results gained in barley by Mariey et al. [51].

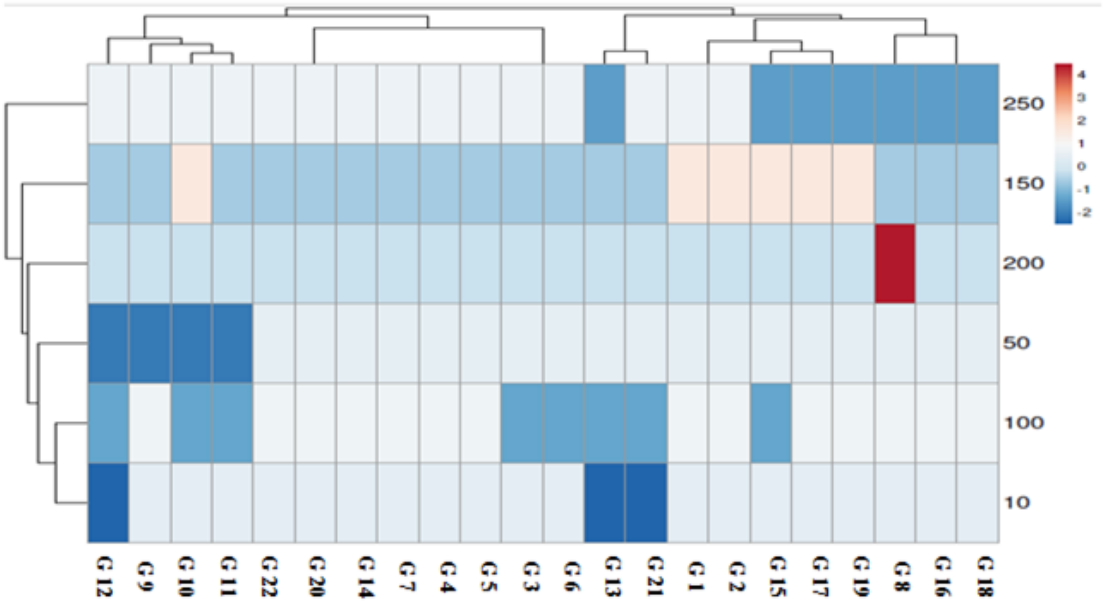


Fig. 4. Multivariate heat-map illustrating the genetic diversity of 22 faba bean landraces, based on the SDS-PAGE for using the module of heat-map of R software

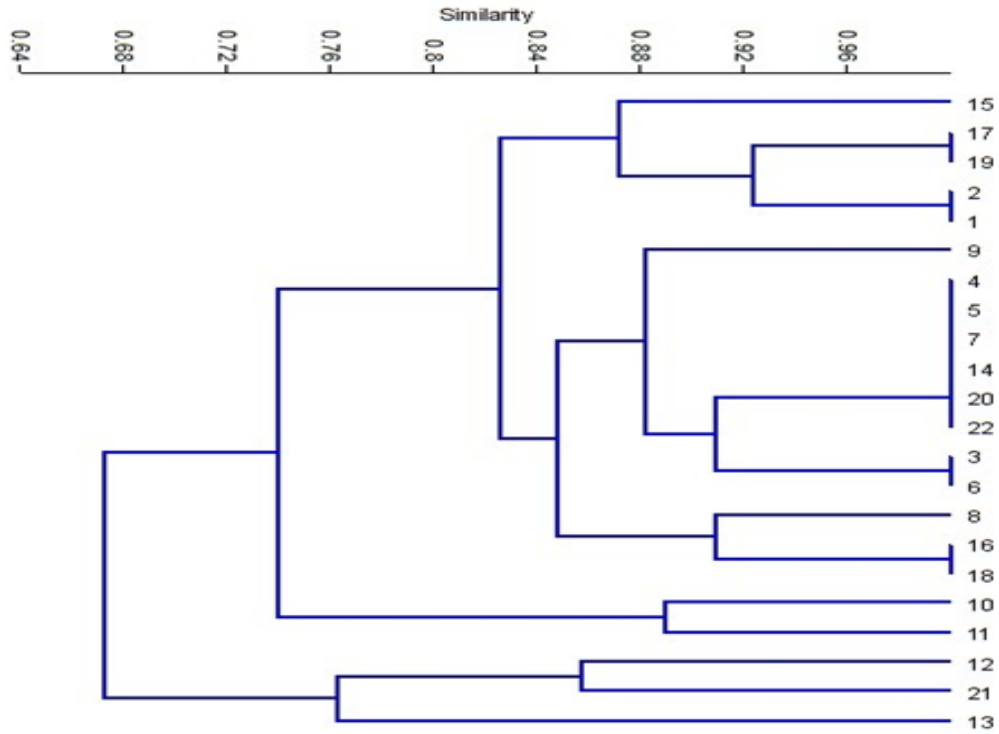


Fig. 5. Cluster tree illustrating the genetic distance among 22 lines from *V. faba* based on the analysis of SDS-PAGE protein analysis using the Euclidean distance and the UPGMA algorithm in the PAST Software

Table 5. Genetic similarity of the 22 lines from *Vicia faba* based on SDS_PAGE

	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**
Line 1	1.00																					
Line 2	1.00	1.00																				
Line 3	0.83	0.83	1.00																			
Line 4	0.92	0.92	0.91	1.00																		
Line 5	0.92	0.92	0.91	1.00	1.00																	
Line 6	0.83	0.83	1.00	0.91	0.91	1.00																
Line 7	0.92	0.92	0.91	1.00	1.00	0.91	1.00															
Line 8	0.77	0.77	0.73	0.83	0.83	0.73	0.83	1.00														
Line 9	0.83	0.83	0.80	0.91	0.91	0.80	0.91	0.73	1.00													
Line 10	0.83	0.83	0.80	0.73	0.73	0.80	0.73	0.55	0.80	1.00												
Line 11	0.73	0.73	0.89	0.80	0.80	0.89	0.80	0.60	0.89	0.89	1.00											
Line 12	0.60	0.60	0.75	0.67	0.67	0.75	0.67	0.44	0.75	0.75	0.86	1.00										
Line 13	0.60	0.60	0.75	0.67	0.67	0.75	0.67	0.67	0.50	0.50	0.57	0.67	1.00									
Line 14	0.92	0.92	0.91	1.00	1.00	0.91	1.00	0.83	0.91	0.73	0.80	0.67	0.67	1.00								
Line 15	0.83	0.83	0.80	0.73	0.73	0.80	0.73	0.73	0.60	0.80	0.67	0.50	0.75	0.73	1.00							
Line 16	0.83	0.83	0.80	0.91	0.91	0.80	0.91	0.91	0.80	0.60	0.67	0.50	0.75	0.91	0.80	1.00						
Line 17	0.92	0.92	0.73	0.83	0.83	0.73	0.83	0.83	0.73	0.73	0.60	0.44	0.67	0.83	0.91	0.91	1.00					
Line 18	0.83	0.83	0.80	0.91	0.91	0.80	0.91	0.91	0.80	0.60	0.67	0.50	0.75	0.91	0.80	1.00	0.91	1.00				
Line 19	0.92	0.92	0.73	0.83	0.83	0.73	0.83	0.83	0.73	0.73	0.60	0.44	0.67	0.83	0.91	0.91	1.00	0.91	1.00			
Line 20	0.92	0.92	0.91	1.00	1.00	0.91	1.00	0.83	0.91	0.73	0.80	0.67	0.67	1.00	0.73	0.91	0.83	0.91	0.83	1.00		
Line 21	0.73	0.73	0.89	0.80	0.80	0.89	0.80	0.60	0.67	0.67	0.75	0.86	0.86	0.80	0.67	0.67	0.60	0.67	0.60	0.80	1.00	
Line 22	0.92	0.92	0.91	1.00	1.00	0.91	1.00	0.83	0.91	0.73	0.80	0.67	0.67	1.00	0.73	0.91	0.83	0.91	0.83	1.00	0.80	1.00

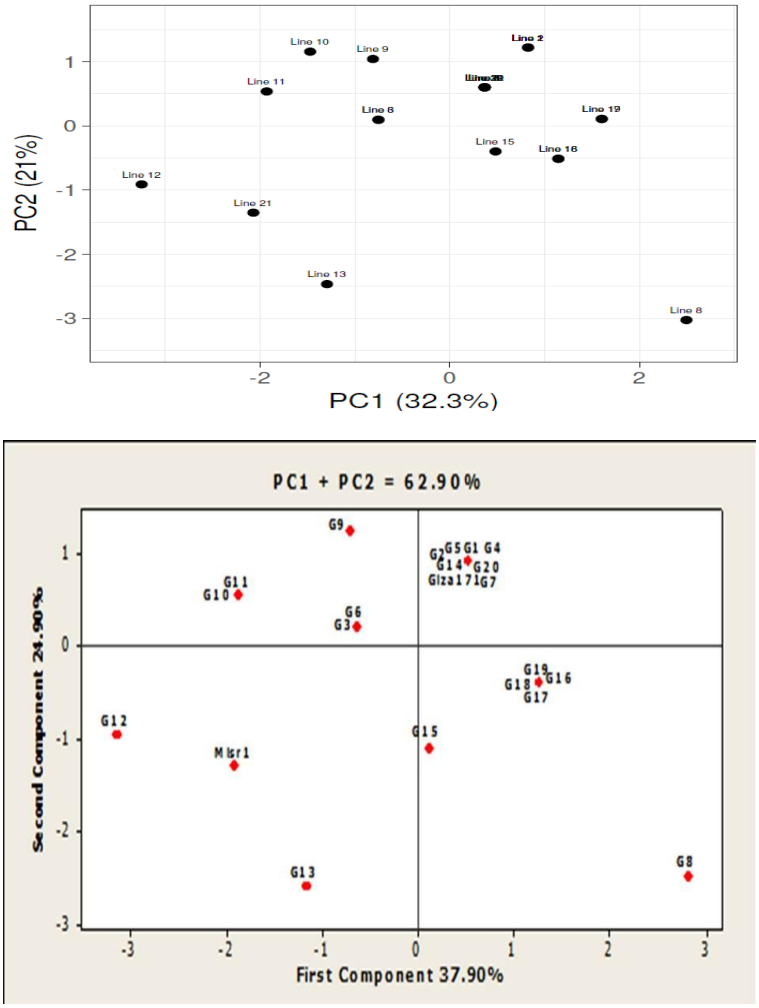


Fig. 6. Principle component analysis (PCA) scatter diagram illustrating the genetic diversity expressed by the grouping of 22 lines based on SDS-PAGE protein analysis and by blotting the first two principal components using PAST software

Landrace comparison (polygon graph):

Combined morphological yield traits and biochemical protein total amplified polymorphic bands [50]; these traits can be used to get two ways (GT) biplot to study the landrace by traits data. This graphical method used to discuss that GT biplot graph was possible to be a good alternative procedure for each of correlation and cluster analyses. Landraces were compared on the basis of multiple traits (morphological and biochemical traits) and to identify landraces that are characterized in certain traits and therefore can be nominee for selection or integrated in faba bean breeding program [30,52].

Polygon graph Fig. (7) illustrated the landrace by trait (GT) biplot, showing which landrace had the highest values for which traits for 22 faba bean landraces. The biplot graph cleared the relationship among the studied faba bean landraces using the seed yield and its related traits with protein total amplified polymorphic bands. The first and two principal components (PC1 and PC2) explained 61.37% and 18.97%, respectively. The first two (PC's) gathered as explained 80.34% of the total variation of the mean performance of the faba bean data in GT biplot. Then, gathered PC's (80.34%) that reflected more than (60%) of the total variation achieved the goodness of fit the GT biplot model Gurmu et al. [53] and Sharifi (2018) in faba bean.. This relatively high proportion reflects the clarity of the relationships among the landraces and the studied traits.

Based on traits, polygon showed that seed yield (SY) was located with number of seeds (Seed), number of pods (Pod) and harvest index (HI) in the same mega environment. Generally, it was mean number of seeds and pods that located on the right side of graph were the most positive effective traits in faba bean seed yield, causing highest harvest index [54]. Meanwhile,

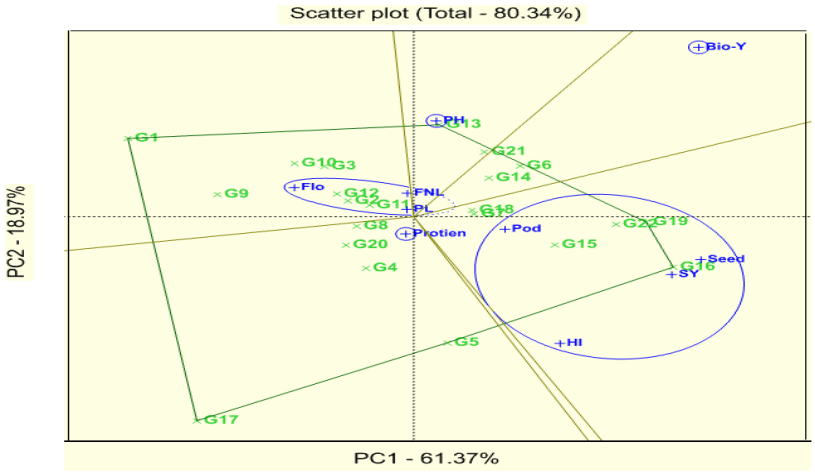
days to flowering (Flo), first node height (FNL) and pod length (PL) that located on the left side of graph were negative correlated with seed yield.

The polygon sides can facilitate comparison between landraces located around the neighboring vertex. It is obvious that landrace G16 and G19 with the highest check Giza716 that located in the same sector recorded the highest values at all in (SY), (Seed), (Pod) and (HI), that reflected to increase these landraces in seed yield, number of seeds, number of pods and harvest index.

Landraces; G12, G2 and G11 were the best landraces in terms of (Flo), (FNL) and (PL) that characterized as early flowering, shortest flowering nods and shortest pod length.

It is noted that the landrace G13 placed into pant height trait (PH) sector recorded the highest plant height. Because the biological yield (Bio-Y) was placed far from all studied traits and landraces, indicating to landraces had poor performance for biological yield trait. Meanwhile, protein amplified polymorphism located near original point, indicating to existence moderate values for this studied trait.

From the previous results mentioned that the current landrace groups G16, G19 and check Giza716 were agreed with those obtained by the cluster analysis. Accordingly, the GT biplot graph is a good preferred alternative procedure for each of correlation and cluster analyses and considered an effective technique beside or instead of cluster analysis for facility the interpretations with information abundance.



PH: Plant height, FNL: First node length, Pod: No. of pods /plant, PL: Pod length, SY: Seed yield /plant, Seed: No. of seeds/plant, Flo: days to 1st flowering, Bio-Y: Biological yield, HI: Harvest index and Protein: total amplified polymorphic bands

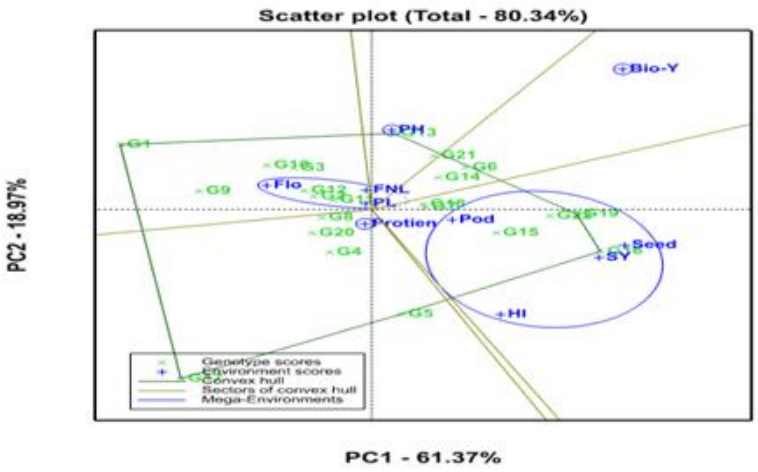


Fig. 7. Polygon view landrace by trait (GT) biplot showing which landrace had the highest values for which traits for 22 faba bean landraces

4. CONCLUSION

Morphological characters and protein marker allow discrimination among the Egyptian faba bean germplasm. Results for the gatherings of faba bean estimated such Polymorphic protein bands analysis might be utilized to genotype accessions from different countries and generate sufficient information to infer as possible source germplasm materials in breeding programs. Results highlighted the presence high genetic diversity. Faba bean landraces as G8, G19, G16, G15 and G2 could consider as the best ones for potential breeding programs to improve desirable agronomical and protein traits. Moreover, several traits were positively correlated with seed yield and one to each other, helping in screening for better faba bean germplasm phenotype. Results showed that there were high genetic information differences among graphical landraces which offered new information about the genetics relationships between Egyptian faba bean which they are useful for germplasm identification and for their utilization in further faba bean programs under different environments.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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