

Estimation of diversity among faba bean accessions based on agro-morphological attributes using multivariate analysis approach

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Faba bean is an important grain legume crop worldwide, and the use of plant genetic breeding resources can help develop high-yield and stress-tolerant plant genotypes. In order to attain optimal outcomes, breeding programs must collaborate with a substantial range of genetic variations. In this study, we evaluated the genetic diversity of 144 faba bean accessions collected from different parts of the world and grown under Saudi Arabian conditions, based on agro-morphological characteristics. We recorded a wide range of variability for all the qualitative characters, except growth habit (determinate 1.3%; indeterminate 98.7%) and pod shattering (non-shattering 99.4%; shattering 0.6%). We found a high level of diversity in qualitative characteristics, including leaflet size, stipule spot pigmentation, flower ground color, the intensity of streak, wing petal colors, pod colors, pod angle, hilum color, pod shattering, ground color of testa and seed shape in the tested faba bean accessions. Based on agro-morphological characteristics, the genotypes were identified as significant for traits including average seed weight, number of branches from the basal node, plant height, pod length, seed weight per plant, number of seeds per pod, seeds per plant, pods per plant, and 100-seed weight. The principal component analysis (PCA) showed that the highest (95.32%) diversity came from three PCs, and the dendrogram analysis divided faba bean accessions into 8 distinct groups based on their origin. The cluster study of the tested faba beans accessions was based on the origin as well as on the genetic history. The extent of variability and diversity observed in the tested faba bean accessions indicates their potential for use in the faba bean breeding program.

Keywords: Genetic diversity, flower color, pod angle, Multivariate analysis.

INTRODUCTION

Faba bean (*Vicia faba* L.) is native to North Africa and West Asia and belongs to the family Fabaceae, subfamily Faboideae. It contains (n=12) diploid chromosome numbers. It is also known as the broad bean, fava bean, field bean, horse bean, and tic bean (Duc, 1997) and is the fourth leading legume crop grown worldwide (López-Bellido *et al.*, 2005). Faba bean contains a higher amount of protein relative to other pulses i.e., beans, lentils, chickpea, and peas (Millar *et al.*, 2019), and is abundant in essential vitamins and minerals and has a low-fat content (L'Hocine *et al.*, 2020). The common bean is known to possess notable antioxidant capabilities, particularly with regard to flavonoids, polyphenols, and phenolics. These phenolic compounds, which possess antioxidant properties, could serve as an excellent source of natural antioxidants for the prevention of chronic diseases and the promotion of good health (Fendri *et al.*, 2022).

During the last few decades, the faba bean crop has been facing many challenges including a lack of improved varieties, a lack of adoption of better production technology, and diseases and insect pest problems (Röös *et al.*, 2020). Lack of genetic diversity and loss of vigor are other major reasons for the stagnant yield potential of existing varieties (Gresta *et al.*, 2010). Plant genetic resources play a very important role in the program of developing elite crop genotypes. However, the lack of proper characterization and evaluation of available germplasm followed by its improper utilization are some of the causes of its declining gene pool vigor. Breeding improvement is only possible if the available genetic resources are characterized for their key traits of interest (Salgotra and Chauhan, 2023). The gene pool serves as the most important crop improvement tool (Nadal *et al.*, 2003; Terzopoulos and Bebeli, 2008).

The availability, conservation and utilization of genetic resources are crucial for crop improvement. Collecting plant genetic resources involves acquiring all available wild and



improved plant genotypes and conserving them using suitable methods such as *in situ* or *ex situ* conservation. To achieve this, the focus is on morphological and molecular characterization of the genetic resources (Rao, 2004). Molecular markers play a significant role in excluding possible errors that can arise in the morphological evaluation of crops due to environmental interactions and are powerful in assessing and discriminating genetic diversity of different crops. Utilization faba bean genetic diversity and selection based on morphological characteristics can help construct selection indices for faba bean improvement (Elshafei *et al.*, 2019). Detection of genetic diversity based on morphological traits is important for identifying and protecting for future utilization.

Saudi Arabia is one of the top importers countries of faba bean. However, the production of this crop in the kingdom is extremely low due to the lack of emphasis on research for

improved varieties, while the per capita demand is increasing (FAO, 2007). The absence of wild ancestors, difficulty in interspecific crosses among faba bean and the availability of only natural or mutagenic lines make it necessary to make dedicated efforts to explore germplasm variability. Hence, the current study aimed to assess the genetic diversity among faba bean accessions available at the King Saud University gene bank collected from different regions based on agromorphological traits using the International Union for the Protection of New Varieties of Plants (UPOV) and International Board for Plant Genetic Resources (IBPGR) descriptors under field condition.

MATERIALS AND METHODS

The experiment was conducted at the Education Research Farm, College of Food and Agricultural Sciences, King Saud University located at “E 24.72°, 46.62° N” classified as a hot desert climate. The monthly maximum and minimum temperatures of the experimental site during the course of investigation are given in Figure 1. The experimental soil was loamy sand with 0.28% organic matter, 12.8 N%, 20.6 ppm absorbable P, and 86.6 ppm absorbable K. The experiment was planted in the field following lattice layout design. The seedbed was prepared by ploughing the experimental soil twice, the field was divided into plots, and ridges were made. Each plot included 6 m long, and 60 cm spaced 6 ridges. During soil preparation, diammonium phosphate (DAP; 18% N; 46% P₂O₅) was applied at 150 kg/ha. Two seeds were manually planted in each hill. Seeds of faba bean accessions were sown on 03 November. All other cultural practices were kept optimum to have the good health and vigor of plants. Hoeing was done after 15 and 30 days during the vegetative growth and irrigation was applied after one week. The crop was harvested at harvest maturity.

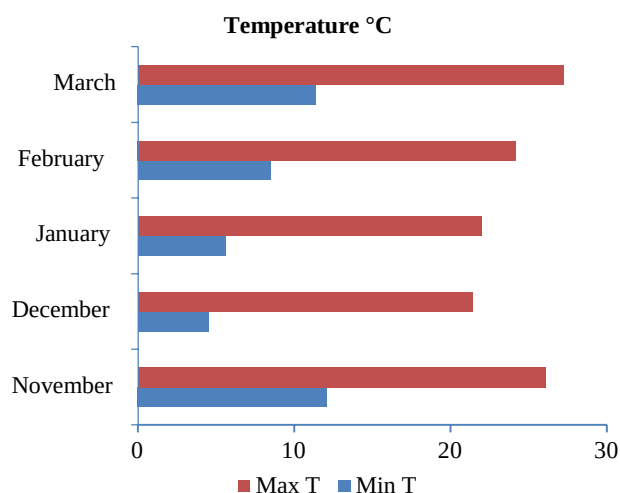


Figure 1. Monthly minimum and maximum temperature recorded during the growing season.

Observations and Measurements: Plant height was recorded at the maturity stage from the bottom to the top of the leaf. The number of branches emerging from the leaf axils on the upper half of the main stem at the physiological maturity stage was noted and recorded as the number of branches from the basal node. The leaflets were graded as large, medium, and small. Stipule spot pigmentation was recorded as absence and presence, and flower ground color was noted as pink, violet and white. The intensity of steaks was measured as intense, moderate, no steak, and slight. The wing petal color was noted as spotted, uniformly colored, and uniformly white. The pod color was recorded as dark brown and light yellow. The pod angle was noted in the form of erect and pendant while pod degree curvature was recorded as medium, strong, very weak, and weak. The hilum color was recorded as black, light yellow and mixed form. The pod shattering behavior was recorded as shattering and non-shattering. The growth habit was recorded as determinate and indeterminate. The ground color of the testa was recorded as black, brown, dark brown, green, grey, light brown, light green, mixed and yellow type. The seed shape was recorded as angular, flattened, and round type. Pod length was measured from the branch tip to the total length of the pod. All pods were harvested from five randomly selected plants and were threshed to record the number of seeds per pod and the number of seeds per plant. A sub-sample of 100 seeds was taken and weighed to record the 100-seed weight. **Statistical analysis:** The experimental data were analyzed using SAS 3.0 and means were compared using the least significant difference test at a 5% probability level. The data were normalized and analyzed using Euclidian distance for principal components analysis (PCA) and a dendrogram was developed with distances using Unweighted Pair Group Method with Arithmetic Mean (UPGMA) in PAST 3.11 (Hammer *et al.*, 2017).

RESULTS

The analysis of variance revealed significant differences among the tested genotypes for morphological characteristics and yield related traits (Table 1). The descriptive statistics results, including mean, minimum, maximum, and standard deviation showed, indicate a higher genetic diversity for both quantitative and qualitative characters in the tested faba bean accessions (Figure 2). The number branches from the basal node showed a diverse range among the tested accessions, with significant differences observed for all studied characters. Accession L56 had the maximum number of branches from the basal node, followed by FLIP06003FB, while the minimum number of branches from the basal node was recorded in the accession Hassawi-3 (Figure 2).

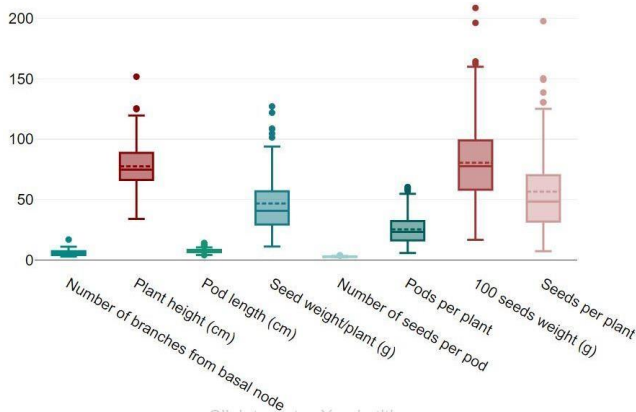


Figure 2. Descriptive statistics for quantitative attributes of the tested faba bean genotypes (Box plot dots represent maximum, upper fence, mean, median and lowest value)

Accession FLIP08057FB recorded the highest plant height, while accession 98930995 had the minimum plant height. The accession FLIP08012FB had the highest seed weight, while the lowest seed weight was recorded in the accession FLIP08005FB. The number of seeds per pod ranged from 2.07 to 3.08, with the lowest value recorded for the accession FLIP08054FB, and the highest number of seeds per pod recorded for the FLIP03011FB. The maximum number of seeds/plant was recorded for the number of seeds/pods FLIP08012FB, while the minimum was recorded for the number of seeds per pod H3 and showed a diverse range

(7.13-197.73). The highest number of pods per plant was recorded for the number of seeds per pod in the accession WRB21, while the lowest was recorded for the Syrian collection. The 100-seed weight also showed good diversity (16.57-208.68) among the tested accessions, with the lowest 100-seed weight recorded in the accession FLIP03004FB and the highest value recorded for accession H8.

The accession L56 had 196% more basal branches than other faba bean accessions. Among the 144 accessions, 11 (FLIP06001FB, FLIP06003FB, FLIP06012FB, FLIP08052FB, WRB15, FLIP03019FB, FLIP03025FB, Pakistani, L56, FLIP08005FB and FLIP08028FB) had a mean performance of 50% or more compared to other (Figure 3a). The five faba bean accessions with the highest plant height were FLIP08057FB, FLIP03001FB, FLIP08016FB, FLIP08017FB and FLIP08018FB. Among the 21 accessions, there were a higher number of pods per plant (Figure 3c). The accession WRB21 had the highest high performance recorded in this group, while FLIP08055FB, FLIP08029FB, and FLIP08027FB also performed well for the number of pods per plant (Figure 3b).

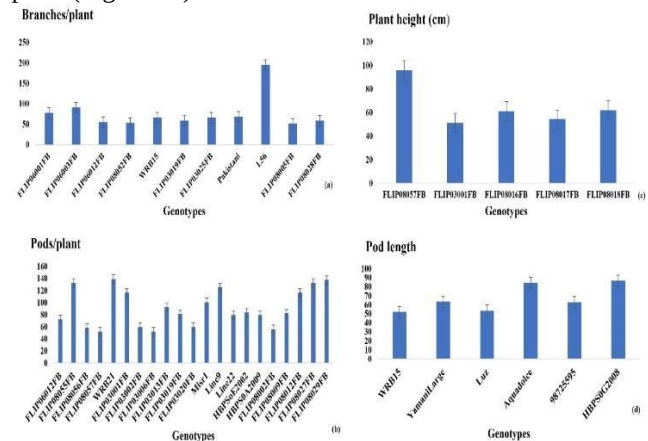


Figure 3. Number of basal branches/plant (a), number of pods/plant (b), plant height (cm) (c) and Pod length (cm) (X-axis showed the genotype names and Y-axis showed the 50% more increased in compression relative to grand mean of all 144 faba bean accessions studied)

The two faba bean accessions with the lowest number of pods per plant among the group of 50% more grand mean results were FLIP08056FB and FLIP03006FB. Data regarding pod

Table 1. Analysis of variance (ANOVA) results of 144 faba bean genotypes based on error mean square (EMS)

SOV	NOB	PH	PL	PP	SPP	SPI	ASW	HSW
Rep	9.77	191.58	1.84	493.51	0.05	29.27	18.32	262.89
Genotypes	13.14**	1051.31**	7.49**	478.66**	0.29**	3506.28**	1679.48**	3321.48**
Error	0.38	96.45	0.16	58.88	0.24	53.82	742.48	36.21

SOV= Source of variation; NOB= Number of branches; PH= Plant height; PL= Pod length; PP= Pods per plant; SPP= Seeds per pod; SPI= Seeds per plant; ASW= Average seed weight; HSW= 100 seeds weight; **= Highly significant at 5% probability value

length was presented for 6 accessions that qualified for 50% more value than the grand mean among 144 faba bean accessions (Figure 3d). Accessions HBPS0G2008 and Aquadolce were the best performers among all the accessions studied, recording 86.52% and 84.65 % high efficiency than all the studied accessions, respectively,

The genotypes FLIP08012FB and HBPS0E2002 showed the highest efficiency (172.27% and 161.09 %) for seed weight relative to other faba bean accessions (Figure 4a). Similarly, the highest increase (249.91%) in the number of seeds/plants was recorded for FLIP08012FB, followed by the variety HBPS0E2002 recorded a 164.03 % increase relative to the grand mean value (Figure 4b).

The highest 100 seeds weight was recorded in accession H8 which was followed by accession H3. The 11 accessions performed better and showed 50% more mean compression value than all other tested accessions (Figure 4c). A great range of variability was observed for all the qualitative characters except growth habit (determinate 1.3%; indeterminate 98.7%) and pod shattering (non-shattering 99.4%; shattering 0.6%). Moreover, seed shape also had less variability, while characters i.e., leaflet size, flower ground color, wing petal color, stipule spot pigmentation, the intensity of streaks, pod angle, hilum color, pod angle, pod degree of curvature, and ground color of testa showed the great extent of variation (Table 2). The mean values of morphological traits for 144 faba bean accessions were presented in Table 1S.

Table 2. Qualitative characteristics of 144 faba bean (*vicia faba* L.) accessions

Variable	Percent	Variable	Percent
Leaflet size		Pod Color	
Small	17.5	Dark brown	26
Medium	46.1	Light yellow	74
Large	36.4		
Flower ground color		Growth Habit	
Pink	46.8	Determinate	1.3
Violet	14.9	Indeterminate	98.7
White	38.3		
Intensity of streaks		Pod shattering	
Intense	56.5	Non-shattering	99.4%
Moderate	9.7	Shattering	0.6%
No Streak	33.1	Pod angle	
Slight	0.6	Erect	66.9
Wing petal color		Pendant	33.1
Spotted	66.2	Ground color of testa	
Uniformly colored	0.6	Black	0.6
Uniformly white	33.1	Mixed	11.7
		Yellow	18.8
Pod degree of curvature		Brown Dark	9.7
Medium	23.4	Brown	16.9
Strong	9.7	Light Brown	16.2
Weak	6.1	Green grey	6.5
Very weak	5.8	Light Green	3.9
Hilum Color		Seed shape	
Black	68.2	Angular	11.0
Light yellow	14.3	Flattened	88.3
Mixed	17.5	Round	0.6

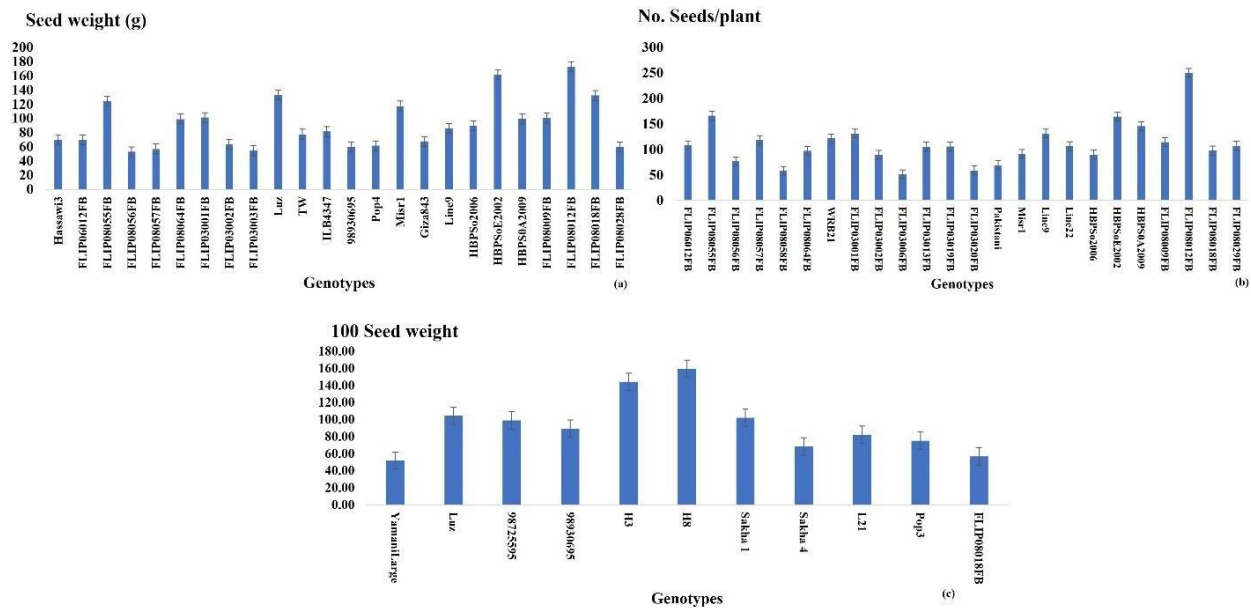


Figure 4. Seed weight (g) (a), number of seeds/plant (b), and 100 seeds weight (g) (c) (X-axis showed the genotype names and Y-axis showed the 50% more increased in compression relative to grand mean of all 144 faba bean accessions studied)

Table 3. Clustering patterns of faba bean accessions based on morphological data.

Source	Number of accessions	Cluster 1	Cluster 2	Cluster 3	Cluster 4	Cluster 5	Cluster 6	Cluster 7	Cluster 8
Australia	1	1							
Egypt	27	5	4	7	5	5			1
ICARDA	85	9		15	21	5	13	8	14
Saudi Arabia	16	2	3	2	6	1		1	1
Sudan	4	1		2		1			
Spain	4	2	1	1					
Yamen	1	0				1			
Pakistan	1	0						1	
England	5	0		1	2		1		1
Total	144	20	8	28	34	13	14	10	17
frequency	100	13.8	5.5	19.4	23.6	9.3	9.7	6.9	11.8

The highest eigenvalue (1716.77) and variance percentage (51.20) were in the first principal component (PC). The first three PCs contributed the highest variance percentage (95.32%) among the studied faba bean accessions. The number of branches from the basal node, plant height, pod length, seed weight/plant, number of seeds per pod, seeds per plant, and pods per plant had the highest contribution in the PC1. However, 100 seed weight contributed more in PC2 and PC3 (Figure 4).

Faba bean accessions clustering patterns of faba bean accessions based on agro-morphological data are given in Figure 5 and Table 3. The cluster analysis grouped the faba bean accessions into eight groups. The Australian faba bean genotype was present alone in cluster I and not sharing any other genotype. The 27 Egyptian accessions were scattered in different groups along with other faba bean accessions. Egyptian (5) accessions were present in Cluster I, 4 in Cluster II, 7 in Cluster III, 5 in Clusters IV and V, and 1 in Cluster VIII. Similarly, 85 faba bean accessions were distributed in 8 clusters. Nine accessions were present in Cluster I, 15 accessions were present in Cluster III, 21 in Cluster IV, 5 were in Cluster V, 13 in Cluster VI, and 8 and 14 were present in VII and VIII clusters respectively. The local (16) Saudi accessions were also scattered in the dendrogram; Cluster I (2), Cluster II (3), Cluster III (2), and Cluster IV (6), and each of Cluster V-VIII contained one faba bean accessions respectively. The collection from Sudan was present in Cluster I (1), Cluster III (2), and Cluster V (1). Similarly, the collection from Yemen and Pakistan contained only 1 accession in each of Cluster V and Cluster VII. However, the collection from England was scattered and was present in 4 groups: Cluster III (1), Cluster IV (2), and Cluster VI (1) (Table 3).

The cluster analysis based on UPGMA for 144 faba bean accessions was presented in Figure 5. The cluster was made on genetic distance value among the faba bean accessions and divided at average distance value. The dendrogram produced by Jaccard's coefficient and the UPGMA clustering method based on agro-morphological data in six faba bean accessions

resources (Figure 5). Based on locations the accessions were categorized into 4 major groups. The first group contained accessions from Egypt and Saudi Arabia; whereas accessions from Spain and Sudan remained separate while accessions from UK and ICARADA were present in the same group (Figure 5).

The clustering patterns of faba bean accessions based on agro-morphological data are presented in Table 3 and Figure 6. Based on nine geographical locations (Australia, Egypt, ICARDA, Saudi Arabia, Sudan, Spain, Yamen, Pakistan, and England), faba bean accessions were divided into eight clusters.

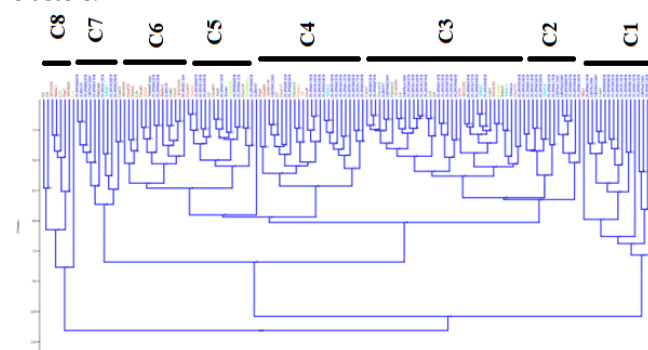


Figure 6. Dendrogram produced by Jaccard's coefficient and the UPGMA clustering method based on morphological data in 144 faba bean accessions. (C represents the cluster and color represents the country in the figure)

The first cluster represents a total of 20 accessions, including 1 from Australia, 5 from Egypt, 9 from ICARADA, 2 from Saudi Arabia, 1 from Sudan, and 2 from Spain. Similarly, the second cluster contains 8 accessions (4 from Egypt, 3 from Saudi Arabia, and 1 from Spain), the third cluster contains 28 accessions (7 from Egypt, 15 from ICARADA, 2 from Saudi Arabia, 2 from Sudan, 1 from Spain, and 1 from England), the fourth cluster contains 34 accessions (5 from Egypt, 21 from ICARADA, 6 from Saudi Arabia, 2 from England), the fifth

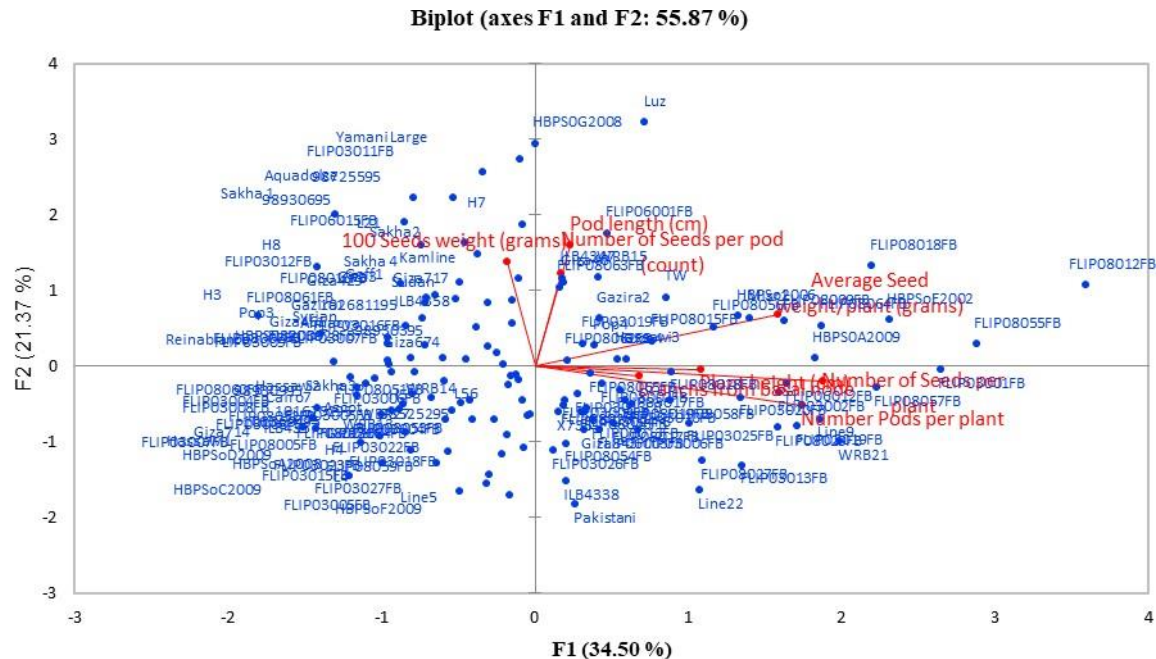


Figure 5. Principal component analysis (PCA) of tested faba bean accessions for different morphological traits.

contained 13 accessions (5 from Egypt, 5 from ICARDA, 1 from Saudi Arabia, 1 from Sudan, 1 from Yemen), the sixth contained 13 accessions (12 from ICARDA, 1 from England), the seventh cluster contained 10 accessions (8 from ICARDA, 1 from Saudi Arabia, and 1 from England) and the eighth cluster contained 17 accessions (1 from Egypt, 14 from ICARDA, 1 from Saudi Arabia, and 1 from England) (Table 3). The cluster analysis was also constructed based on larger resource collections (Saudi Arabia, Egypt, Spain, Sudan, ICARDA, and the UK) using UPGMA clustering methods. Two distinct groups were formed. In the first group Saudi Arabia, Egypt, Spain, and Sudan were present in one group, however, ICARDA and UK collections were present in the second group representing the clustering based on their origin and showed similarity among the faba bean accessions based on their agro-morphological characteristics (Figure 1S).

DISCUSSION

Genetic diversity and its evaluation are considered the first step in plant breeding programs. A high level of genetic diversity in plant populations increases the chance of finding and using the desirable strains in breeding. Moreover, knowledge of genetic diversity is essential for mapping associations, genomic selection, and classification into different accession groups (Solomon *et al.*, 2019). In this study, the variance analysis revealed substantial differences among the tested genotypes for morphological and yield related traits. The selection of agronomic traits based on high

variance values can be utilized effectively for direct selection and inclusion of accessions with desirable traits in the breeding systems (Malik *et al.*, 2014). The difference in yield related traits, as noted in this study, indicates a great potential for these accessions use in breeding programs (Malik and Kumari, 2010).

The descriptive results, including mean, minimum, maximum, and standard deviation indicated a higher genetic diversity for both quantitative and qualitative characters in the tested faba bean accessions (Figure 2). Similar levels of genetic diversity were observed in other legumes, which suggests that breeding efforts have mainly emphasized traits such as plant form, shape, grain and flower color, seed coat and pod color, earliness, pest and disease resistance, 100 seed weight, primary branches, 50% flowering, pod size, pod number and harvest index (Buleti *et al.*, 2020).

Plant genetic resources are essential for global food security, in the form of genetic material diversity performed differently under different environmental conditions (Smale and Jamora, 2020). The availability, conservation, and utilization of genetic resources are critical for crop improvement. Successful examination of the genetic material, conservation, and utilization of genetic resources requires appropriate *in situ* or *ex situ* conservation methods for all available wild and newly developed cultivars (Wambugu and Henry, 2022). Two accessions, FLIP08056FB and FLIP03006FB (ICARDA), had the lowest number of pods per plant compared to other accessions in the study whereas six accessions had pod length at least 50% higher than the grand

mean of 144 faba bean accessions. The accessions BPS0G2008 (ICARDA) and Aquadolce (Spain) were the best performers with high efficiency compared to all the other tested accessions. Similarly, the highest 100 seed weight was recorded in accession H8, followed by Two accession H3 (Saudi Arabia).

Khadivi-Khub & Anjam (2014) suggested that genetic diversity assessed at the population level may not be appropriate for clustering at the regional level because accessions can differ in genetic diversity even within a single population. In addition, accessions can also have different genetic histories from the same origin. Therefore, this is desired to collect, evaluate, and preserve germplasms for potential usage, based on both inter-and intra-regional diversity assessments. However, environmental factors can affect certain traits (Govindaraj *et al.*, 2015).

Seed yield is the most important factor contributing to the variability of the accessions. In this study accessions FLIP08012FB and HBPS0E2002 (ICARDA) had the highest efficiency for seed weight compared with other faba bean accessions, and accession FLIP08012FB (ICARDA) had the highest number of seeds/plants, followed by accession HBPS0E2002 (ICARDA). In another study, Sharifi (2015) opined that yield contributing traits should be considered in faba bean breeding programs as selection criteria. Collecting high-yielding accessions can further leverage this important correlation (Afzal *et al.*, 2018).

The PCA provides grouping information, enabling breeders and researchers to perform unique breeding programs by identifying relevant characteristics (Sharifi *et al.*, 2018). The PCA had been effective in explaining genetic diversity based on agro-morphological traits in chickpea (Tesfamichael *et al.*, 2015) and faba bean (Rebaa *et al.*, 2017). The current study grouped the faba bean accessions into eight clusters according to their origin. A crossbreeding system that selects accessions based on desirable traits may yield high-quality seeds and improve segregates relevant to a setting (Bohra *et al.*, 2022). In addition, accessions are chosen from different groups and a core collection is assembled to conserve the germplasm and exploit genetic diversity for further breeding. Genetic diversity-based conservation will aid in controlling the germplasm resources (Sharifi *et al.*, 2018) in faba beans and will aid in crop improvement programs (Nguyen *et al.*, 2004).

Conclusion: the 144 tested faba bean accessions exhibited a significant degree of diversity in both quantitative and qualitative characteristics. Significant variations were observed among different faba bean accessions collected from different regions worldwide, which could aid in the identification of high-yielding accession suitable for direct use as a new variety or for inclusion in breeding programs in the Kingdom of Saudi Arabia. Based on agro-morphological characteristics, the following genotypes were identified as significant: average seed weight (FLIP08012FB), number of

branches from the basal node (L57), plant height (FLIP08057FB), pod length (HBPS0G2008), seed weight per plant (FLIP08012FB), number of seeds per pod (FLIP03011FB), seeds per plant (FLIP08012FB), pods per plant (WRB21), and 100-seed weight (H8). The chosen faba bean genotypes displayed notable dissimilarities and demonstrated superiority in most of the studied characteristics and need further evaluation for utilization in the breeding program which could lead to improved crop yield and quality characteristics.

Conflict of interest statement: The authors declare that there is no conflict of interest.

Author contributions: SSA conceived the idea, designed the experiment, and supervised the work. MA conducted the experiment, analyzed the data, and prepared the first draft. MF improved and finalized the manuscript.

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