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Molecular Characterization, Chemical Composition, and Descriptive Morphology of Faba Bean Genetic Resource Groups

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FOURTEEN landraces out of the 18 faba bean genotypes comprised the plant materials were used in the present study. PCR-based markers, such as CDDP and SCoT, were used implicated to determine the relationships and phylogenetics of the faba germplasm. The diversity (*h*) averaged 0.33 in total, oscillating from 0.17 to 0.38 for SCoT, with an average of 0.31, and from 0.234 to 0.448 for CDDP, with an average of 0354. The Shannon index (*I*) revealed a grand average of 0.48, with a mean of 0.52 for CDDP, and 0.464 for SCoT. A grand average of 1.564 was found for the effective number of alleles (Ne), which varied from 1.506 (SCoT) to 1.63 (CDDP). The chemical composition of all the germplasm was determined. The protein content ranged from 23.083 % to 35.857 % in the tested Genotypes. L245 had the highest protein content (35.857 %), followed by Sakha 4 (33.633 %). Moreover, this difference also appeared in the heatmap as the reason for the emergence of L82, L221, L217, ALB, L224, Sakha 1, L258, and L253 among the L259 accessions and cultivars. Likewise, the PCA-associated loading plot's biplot revealed that, while the SCoT and CDDP markers Ash %, crude crud fiber, pod no/node, protein %, pod length and seed no/pod traits were positively correlated with L 239, L 245, L 180, L 258 and L 221, respectively; seed weight per plant; 100 seed weight; end flowering date; first flowering date; and fruiting date were associated with the Nubria 1 cultivar.

Key words: Vicia faba, CDDP and SCoT marker, chemical composition, morphological traits.

Introduction

The faba bean, a diploid outcrossing species (2n = 12), has six chromosomes and a "have being genome"10 of over 13 gigabases (Gb). The faba bean genome is extremely conserved in transposable element complex assembly, hence mapping has been based on cloning. Using morphological markers like isozymes, restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD), sequence characterized amplified regions (SCARs), intron targeted amplified polymorphisms (ITAPs), simple sequence repeats (SSRs), and low-density single-nucleotide polymorphism (SNP) markers, the majority of linkage maps so far have had medium saturation (Carrillo-Perdomo *et al*., 2020 and Jayakodi *et al*., 2023).

The SCoT and SRAP methods were used for molecular profiling. The DNA was extracted from fresh leaves, and the PCR techniques employed ten SRAP primers and six SCoT primers. Of the 72 loci produced by SCoT and SRAP-PCR, 17 were monomorphic and 55 were polymorphic. There were 48 total loci in SCoT and 24 in SRAP. According to Essa *et al*. (2023), the average polymorphism (%) values obtained using SRAP and SCoT were 80% and 70.93%, respectively.

Three of the RAPD primers were found to be more appropriate for examining genetic variation among genotypes due to their greater resolving power (RP) rates. Each primer's average resolving power for the SSR markers was 5.24. Additionally, the primer GA4 was determined to be the most suitable for analyzing genetic diversity among the genotypes under investigation due to its greatest RP value

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(7.64). According to the RAPD and SSR data, the average polymorphic information content values for each primer were 0.27 and 0.34, respectively (Dora *et al*., 2017).

Using molecular markers such as RAPD techniques (Abd EL-Mageed., 2018) and ISSRs and SCOTs markers (Ramadan *et al*., 2025) to identify the variations and facilitates the selection of distinguished genotypes which have useful traits in breeding programs, the molecular analysis is more efficient method for estimating genetic diversity because it's not affected by environment, fast, more accurate and doesn't need earlier pedigree information which can improve the efficacy of molecular breeding practices (El-Orabey., *et al* 2020)

The two primary goals of faba bean breeding are to increase seed quality and resistance to abiotic such as drought (Abdelaal 2023) and biotic stresses like parasitic weed plants broomrape (Salman *at al*., 2024). Regarding important quality attributes, gene discovery has led to improvements in the reduction of vicin-convecin tannins and the seed coat, which are the primary anti-nutritional characteristics limiting the use of faba bean seeds. Significant advancements are being made to boost interest in faba beans, despite the fact that genomic resources are still comparatively less developed than those for other legume species (Khazaei *et al*, 2021).

An excellent source of fats, carbohydrates, and protein is faba beans. Bioactive compounds, including polyphenols, flavonoids, vitamins, and minerals, are abundant in beans. There is numerous health benefits associated with these polyphenolic compounds. It is a good option for curing protein deficiency because of its balanced amino acid composition. Faba beans with discoloration, wilting, infestation, and breaking are of poor quality. On the other hand, cooked beans with high hydration and swelling contents are preferred by most processors and consumers because they yield a greater quantity of these materials (Chaudhary *et al*., 2022).

The gastrointestinal tract's release of faba bean peptides after digestion has anti-inflammatory, anti-hypertensive, anti-cholesterolemic, antidiabetic, and antioxidant properties, suggesting that these leguminous crops have a great potential for use as functional foods to help combat the rising incidence of non-communicable diseases (Martineau-Côté *et al*., 2022). Around the world, there are numerous faba bean cultivars that can withstand both biotic and abiotic stressors. According to Maalouf *et al*. (2019), the average return gains vary from 1.65% to 4.17% every year.

The expected genetic gains result from selecting the top 5% of genotypes, with the average percentage ranging from 0.49% to 145.83% (Bullo Neda *et al*., 2021). High genetic progress as a percentage of the average of most qualities was found to be associated with high heritability, suggesting that these features were improved *via* simple selection (Bullo Neda *et al*., 2021, El-Dawy *et al*., 2021). On the other hand, the traits are inner peace length, plant height, number of pods per plant, number of seeds per pod, seed yield per plot of land, and weight of thousands of pods (Bullo Neda *et al*., 2021, El-Dawy *et al*., 2021). Seeds and traits, i.e., inner peace length, seed yield per plot, and weight of thousand seeds, should also be taken into consideration when selecting methods to improve grain productivity in faba beans (Bullo Neda *et al*., 2021; El-Dawy *et al*., 2021; and Nurmansyah *et al*., 2020). Ultimately, this study aims to clarify the molecular characterization of the faba bean using the molecular markers start codon targeted (SCoT) and conserved DNA-derived polymorphism (CDDP) and to perform chemical analysis and obtain a morphological description of the faba bean genetic resource collections (Mahdy *et al*. 2024).

Multivariate analytical techniques that analyze multiple measurements for each genotype at the same time are widely used in the analysis of genetic diversity. Cluster analysis is a valuable biometrical tool aimed at quantifying the degree of genetic divergence among tested genotypes based on their performance and their contributing characteristics. Classification of genotypes according to agronomic traits made using multi-factor techniques can shorten the period and the cost of improving the yield. The presence of genetic variety among plant materials represents the basis for developing an effective breeding program. Information about the nature and degree of genetic diversity helps the plant breeder in choosing diverse parents (Polignano *et al*., 1989), Shadakshari *et al* (2011), Sheykhi *et al*. (2014) and Pallavi *et al* (2020). The objectives of this work were to study the genetic parameters and identify the interrelationships among seed yield and its related characters using correlation and cluster analysis. The results may be helpful in planning appropriate selection strategies for improving seed yield in faba beans.

Materials and Methods

Plant material

The plant materials used in this study consisted of a total of 18 accessions of Faba bean, 14 of which were landraces obtained from the Genetic Resources Research Department (GRRD), which is the gene bank of Bahteem, and four of which were released varieties obtained from the Food Legumes Research Department (FLRD), Field Crops Research Institute (FCRI) Agricultural Research Center (ARC) Giza, Egypt. The origin, names, and numbers of accessions are displayed in (Table 1). The accessions were sown in the Ministry of Agriculture districts within the second and third weeks of October for the two main cropping seasons.

Extraction of DNA

High-quality DNA was extracted from fresh leaves (100 mg) according to the manufacturer's protocol for the ZR Plant/Seed DNA DNeasy Plant MiniPrep™ Kit (www.zymoresearch.com) and then quantified with a NanoDrop Spectrophotometer (Thermo Fisher Scientific, Inc.). The concentrations of the samples were adjusted to 10 ng/µl for subsequent analyses.

PCR-Based Polymorphism

Two molecular markers were used: the start codon target (SCoT) and conserved DNA-derived polymorphism (CDDP), as listed in (Table 2). The twelve CDDP and ten SCoT Primers were synthesized according to Collard and Mackill (2009 a&b).

PCR conditions

PCR reaction was performed in a 25 µl reaction mixture containing 25 ng of template DNA, 0.2 µM dNTPs, 1 µM of each primer, 1.5 mM $MgCl_2$, 1x PCR buffer, and 1 U of Go-Taq Flexi polymerase (Promega). PCR amplification was programmed at 94°C for 5 min as an initial denaturation cycle, followed by 35 cycles of 94°C for 1 min, 50°C for 1 min, and 72°C for 90 s, with a final extension at 72°C for 7 min. The products were resolved via electrophoresis on a 1.5% agarose gel containing ethidium bromide (0.5 µg/ml) in 1x TBE buffer. A 100 bp plus DNA Ladder was used as a molecular size standard. With the aid of a Gel Doc XR™+ System and Image Lab™ software (Bio-Rad®), PCR results were visualized under UV light and photographed.

Data Scoring and Data Analysis

Clear amplicons were selected for statistical analysis. Only amplicons that were obviously polymorphic were scored as present (1) in the data matrix. Bands without clear polymorphisms or that were polymorphic in only one replicate were scored as absent (0). The number of total bands, unique bands, and polymorphic bands and the percentage of total bands that were polymorphic or specific were calculated. Several genetic parameters were estimated, such as Shannon's information index (I) = $-1 \times (p \times \mathrm{Ln}(p) + q \times \mathrm{Ln}(q))$, the effective number of alleles (N_e) = $1 / (p^2 + q^2)$, the diversity (h) = $1 \div (p^2 + q^2)$, and the unbiased diversity ($_uh$) = $(N \div (N - 1)) \times h$), where p = Band Freq. and $q = 1 - p$. The data sets were fed into the GenAlEx (version 6.5) – Genetic Analysis in Excel (Peakall and Smouse, 2006) and SPSS software (version. 14.0).

Chemical composition estimation

Seed sample from each accession (50g) were used to estimate the moisture, ash, crude protein, and crude fiber levels in eighteen different types of faba beans were measured using the techniques outlined by the Association of Official Agricultural Chemists (AOAC 2010). The amount of carbohydrates was determined by using the difference method.

Morphological Traits, Field Experimental, and Field Traits

Design Two field experiments were carried out at the Bahteem Agricultural Research Station (Bahteem, Egypt) during two successive winter seasons to study the morphological traits of the different accessions and cultivars. To differentiate between the studied accessions and cultivars based on morphological characteristics, the following parameters were recorded: days to 50% heading (HD), days to 50% maturity (MD), grain filling period (GFP) (days), plant height (PH) (cm), pod length (PL) (cm), number of seeds per pod, number of pods per plant, and weight of 100 seeds (g). The seed filling period (SFP) was calculated using the following formula: grain filling period = maturity days − flowering day. A randomized complete block design (RCBD) with four replications was used. The plot size was 4 rows that were 3 m long and 20 cm apart. Analysis of variance and least significant difference (LSD) at 5% were used for comparisons between the Genotypes.

The model of cluster analysis was performed for the field traits on genotypes as "r" matrix using a measure of similarity levels and Euclidean distance (Everitt, 1993 and Eisen *et al*., 1998).

Table 1. List of 18 faba bean (*Vicia faba* L.) genotypes.

ID	Accessions	Pedigree	Origin	Description	References
1	L82	Unknown	Qena18 km north Arment, Egypt	Landraces	Elshafei et al., 2019
2	L217	Unknown	Qena—30 km south Qena,Egypt	Landraces	Elshafei et al., 2019
3	L221	Unknown	Sohag—8 km north Sidfa,Egypt	Landraces	Elshafei et al., 2019
4	L224	Unknown	Assiut—Dairut, Egypt	Landraces	Elshafei et al., 2019
5	V 239	Unknown	Sohag—El Bolyoul, Egypt	Landraces	Elshafei et al., 2019
6	L245	Unknown	Minya—12 km west El Minya, Egypt	Landraces	Elshafei et al., 2019
7	V 248	Unknown	Qena2 km northwest west of Naga Hammadey, Egypt	Landraces	Elshafei et al., 2019
8	L251	Unknown	Assiut, Bani Rafe, Egypt	Landraces	Elshafei et al., 2019
9	L252	Unknown	Minya, Beni Mazar, Egypt	Landraces	Elshafei et al., 2019
10	Sakha 4	Line 81/35/2001 Sakha 1X Giza 3	Egypt	Disease Resistance Variety	Amer et al., 2014
11	A.L.B.	Unknown	Individual plant selection in Alegria	Landraces	
12	Giza 3	Giza 1 x Dutch 29 (Introduction)	Egypt	chocolate spot resistance Variety	Amer et al., 2014
13	Nubaria 1	ILB1270 Individual plant selection from Spanish variety	Egypt	Large seeded Variety	Abdalla et al., 2020
14	Sakha 1	Giza 716 X 620/ 283/ 85	Egypt	Disease Resistance Variety	Amer et al., 2014
15	L253	Unknown	Ismaelia—15 km west Zagazig	Landraces	Elshafei et al., 2019
16	L258	Unknown	Zagazig—3 km west Zagazig	Landraces	Elshafei et al., 2019
17	V 259	Unknown	Elsharkya—6 km west Fagus	Landraces	Elshafei et al., 2019
18	V 180	Unknown	Dakhlia—5 km west mitgammer	Landraces	Elshafei et al., 2019

Table 2. Names and sequences of the **CDDP** and **SCoTs** primers.

No.	CDDP	Sequence	SCoT	Sequence
1	MADS-2	ATGGGCCGSGGCAAGGTGG	SCoT-02	ACCATGGCTACCACCGGC
2	ABP1-1	ACSCC**S**ATCCACCGC	SCoT-06	CAATGGCTACCACTACAG
3	ERF1	CACTACCGCGGSCTSCG	SCoT-09	ACAATGGCTACCACTGCC
4	MADS-1	ATGGGCCGSGGCAAGGTGC	SCoT-11	CAACAATGGCTACCACCG
5	Myb1	GGCAAGGGCTGCCGC	SCoT-13	ACCATGGCTACCACGGCA
6	ERF2	GCSGAGATCCGSGACCC	SCoT-28	CAACAATGGCTACCACCC
7	WRKY-R3B	CCGCTCGTGTGSACG	SCoT-45	ACCATGGCTACCACCGAG
8	WRKY-R1	GTGGTTGTGCTTGCC	SCoT-48	CACCATGGCTACCACCAG
9	WRKY-R3	GCASGTGTGCTCGCC	SCoT-49	ACCATGGCTACCACCGTG
10	KNOX-2	CACTGGTGGGAGCTSCAC	SCoT-12	CAACAATGGCTACCACCG
11	WRKY-F1	TGGCGSAAGTACGGCCAG		

Results and Discussion

Molecular Polymorphism Analysis

PCR-based markers, such as CDDP and SCoT, were used to screen for significant polymorphisms and determine the relationships and phylogenetic of the faba germplasm, as shown in (Figure 1). This may be due to the ease of application and abundance of these materials.

Genetic parameters are efficient for distinguishing markers used in discriminatory objects based on polymorphisms. The number of genetic parameters was estimated to evaluate the informative and discriminatory power, as shown in **Table 3**. The diversity (*h*) averaged 0.33 in total, oscillating from 0.17 (SCoT-45) to 0.38 (SCoT-06), with an average of 0.299 for SCoT, and from 0.234 (Myb1) to 0.448 (WRKY-F1), with an average of 0354 for CDDP. The Shannon index (*I*) revealed a grand average of 0.485, ranging from 0.356 to 0.640, with an average of 0.52 for CDDP, and from 0.276 to 0.561, with a mean of 0.464 for SCoT. The effective number of alleles (N_e) ranged from 1.506 (SCoT) to 1.63 (CDDP), with a grand average of 1.564.

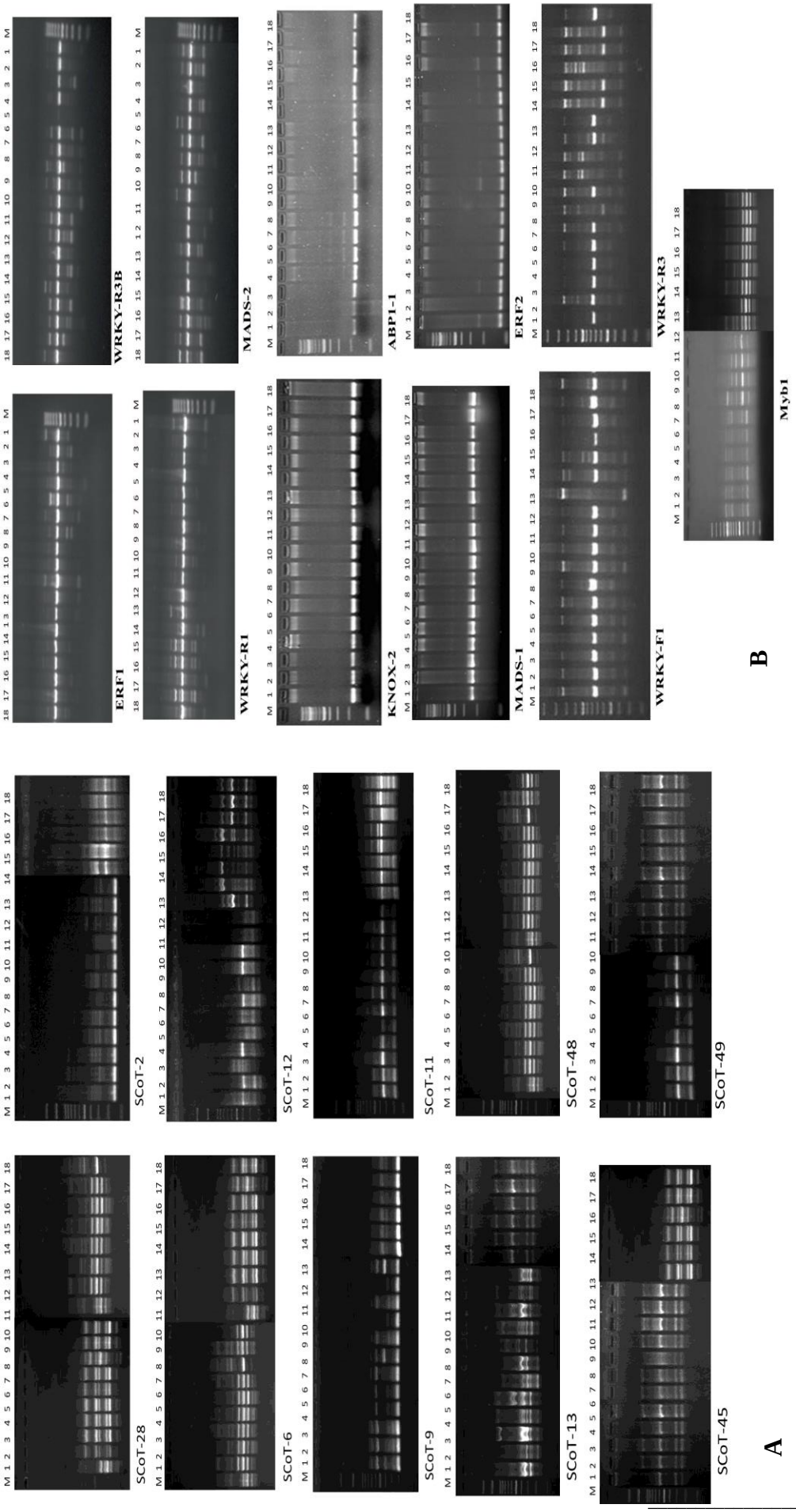

Fig. 1. Electrophoretic profiles of PCR-DNA products using ten SCOT primers (A) and eleven CDDP primers (B) with eighteen faba bean genotypes M: DNA ladder markers ranged in size from 1000-100 bp.

Table 3. Genetic parameters estimated by SCoT and CDDP markers.

Primer	N_L*	N_e	*I*	*H*	$_uh$
SCoT-02	6	1.550	0.505	0.333	0.342
SCoT-06	6	1.667	0.561	0.380	0.391
SCoT-09	5	1.600	0.545	0.363	0.373
SCoT-11	6	1.354	0.364	0.231	0.237
SCoT-13	5	1.552	0.505	0.333	0.342
SCoT-28	5	1.262	0.307	0.183	0.189
SCoT-45	5	1.269	0.276	0.171	0.176
SCoT-48	5	1.562	0.484	0.327	0.336
SCoT-49	6	1.525	0.471	0.316	0.325
SCoT-12	7	1.593	0.527	0.351	0.361
SCoT	**56**	**1.493**	**0.455**	**0.299**	**0.307**
MADS-2	7	1.618	0.527	0.356	0.366
ABP1-1	7	1.737	0.606	0.417	0.429
ERF1	8	1.813	0.629	0.440	0.453
MADS-1	5	1.597	0.484	0.334	0.343
Myb1	7	1.381	0.356	0.234	0.241
ERF2	7	1.561	0.463	0.317	0.326
WRKY-R3B	7	1.465	0.392	0.265	0.273
WRKY-R1	7	1.562	0.484	0.327	0.336
WRKY-R3	6	1.580	0.438	0.309	0.318
KNOX-2	7	1.784	0.625	0.435	0.447
WRKY-F1	6	1.822	0.640	0.448	0.461
CDDP	**74**	**1.629**	**0.513**	**0.353**	**0.364**
Grand	**130**	**1.564**	**0.485**	**0.327**	**0.336**

N_L = no. of locus, N_e= no. of effective allele, I= Shannon's Information Index, h= Diversity, and $_uh$=unbiased diversity.

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The percentage of polymorphism (%P) and genetic fidelity estimated by SCoT and CDDP markers were calculated (**Table 4**). The genetic fidelity of *in vitro* regenerated plants was evaluated using SCoT and CDDP molecular markers. A 23.59% difference was detected between both markers. The SCoT marker revealed a higher percentage of polymorphism than did the CDDP marker. A grand percentage of polymorphisms was estimated at 86.92%, across 130 bands. 7The SCoT marker produced 56 bands across ten primers, with a mean of 91.89% polymorphism. The CDDP markers produced 74 bands across twelve primers, with an average of 87.84% polymorphism. The quality was screened with SCoT and CDDP markers, which exhibited unique banding patterns among the genotypes. Both markers revealed 3 unique bands (Lu) derived from 2 unique bands of SCoT and one unique band of CDDP (**Table 4**). On the other hand, the banding pattern of the PCR-amplified product was found to be monomorphic with most of the primers tested.

Table 4. Polymorphism percentage and genetic fidelity of both markers were used.

Marker	*%P*	L_n	L_u	L_m	L_p	Fidelity
SCoT	91.89	56	2	8	46	23.59
CDDP	87.84	74	1	9	64	
Total	86.92	130	3	17	110	

L_n= number of total bands, L_u= Unique bands, L_m= monomorphic bands, L_p= polymorphic bands.

The genotypes were subjected to molecular polymorphism analysis to confirm the genetic fidelity of the genotypes. The fidelity of the plants was assessed using the CDDP and SCoT molecular markers, and 23.59% polymorphism was detected, possibly due to high genetic diversity among the germplasm. The genetic fidelity of the genotypes was confirmed using SCoT and CDDP molecular markers. The procedure described herein will be feasible for effective genetic improvement of faba beans. Molecular markers, which include Kalanchoe (Al-Khayri *et al*. 2022), *Bacopa monnieri* (Faisal *et al*. 2018), *Ficus carica* (Abdolinejad *et al*. 2020), *Salix lapponum* (Parzymies *et al*. 2020), and *Brassica juncea* (Faisal *et al*. 2021), have been used to evaluate the genetic fidelity of plants.

The polymorphism information content (PIC) score had a grand average of 0.325, oscillating from 0.305 (SCoT) to 0.345 (CDDP). These values revealed that those values are reasonably informative markers and correspond to those of Mahdy *et al*. (2021) and Ghazzawy et al. (2021). Using genetic characterization, Heiba *et al*. (2022) selected promising crosses of four parents for genetic improvement of faba bean. Mahdy and Rizk (2023) highlighted additional insights into the adaptability of dominant species gene pools, which may be utilized in genetic improvement. Diversity (*h*) is the majority of important PICs and provides a high informative marker value which reflects the level of heterozygosity. The h value of a marker with many amplicons desirable for variation splits into three main classes based on Botstein *et al*. (1980). The values are hold more than 0.5 for highly informative markers, a range of 0.25-0.5 for reasonably informative markers, and less than 0.25 for slightly informative markers. The difference in mean heterozygosity (h) between markers and within each marker was due to reason(s), which might undoubtedly mirror inbreeding or selection methods against heterozygotes. The nature of the markers used might be due to the level of observed heterozygosity, which results in the non-detection of homozygotes from heterozygotes because of the presence of null alleles. The heterozygosity represents the direct count of heterozygosity in the population and is estimated based on the allele frequency of individuals given that population according to the Hardy–Weinberg equilibrium. The Shannon information index (I) is one of the major genetic diversity indices used (Sherwin *et al*., 2006). The effective number of alleles (N_e) is a reciprocal of gene homozygosity (Hartl & Clark, 1997). The N_e is used to corollary h; when h is high, N_e will be the highest.

Heatmap and PCA Analysis

The genetic diversity parameter data revealed by the SCOTs and CDDP markers were utilized to calculate the genetic diversity of the studied genotypes by using multivariate clustering, principal component analysis (PCA), and heatmap analyses. Multivariate compound similarity analysis is usually utilized to determine the genetic variance of plant breeds, which is detailed in heatmaps (Mohamed *et al*., 2021). The multivariate compound similarities were presented as a heatmap constructed using R software. As indicated by the columns, 18 faba bean accessions and cultivars were clustered into 3 clusters of at least 2 per cultivar (Figure 2). The first cluster included the Sakha 1, Nubria 1, Giza 3 cultivars and the ALB, L217, L82 and L253 accessions. The cultivar Sakha 4 and the L252, L251, V 248, L245 accessions were discriminated as two neighboring pairs of genotypes. The third cluster consisted of the V 180, V 259, L224, L221, V 239 and L258 genotypes.

In a PCA scatter plot, the SCoTs and CDDP markers reflect the robustness of the markers in categorizing the investigated cultivars and accessions. PCA analysis indicated that the six accessions and the cultivars: Sakha 4, L258, V 248, L252, L251 and V 239 were distinct from the other accessions and cultivars (Figure 3). Neighboring affinity was also apparent between the L221, L245, V 180 and V 259 accessions. Conversely, the remaining cultivars: Nubria 1, Sakha 1, Giza 3, and the ALB accession were scattered at some distance from one another. The accessions L253, L82 and L217 were the best foragers according to cluster analysis, which also indicated that there was a significant distance between Sakha 4 and Sakha 1 and between Giza 3 and Nubria 1.

The differences among the studied accessions in terms of geographical location, cultivar release and pedigree may be due to previous alterations in morphology and production conditions. These morphological characteristics and geographical location may increase or decrease the genetic variation between cultivars. The data from the SCoT and CDDP markers analyzed in this study might be explained by the instability of total number of band's insertion events, accession and cultivar production, and behavior under environmental conditions (Mohamed *et al*., 2021 and Omar *et al*., 2023). There may be a correlation between the high degree of polymorphism observed in SCoTs and CDDP markers and genotype diversity (Mohamed *et al*., 2021 and Omar *et al*., 2023).

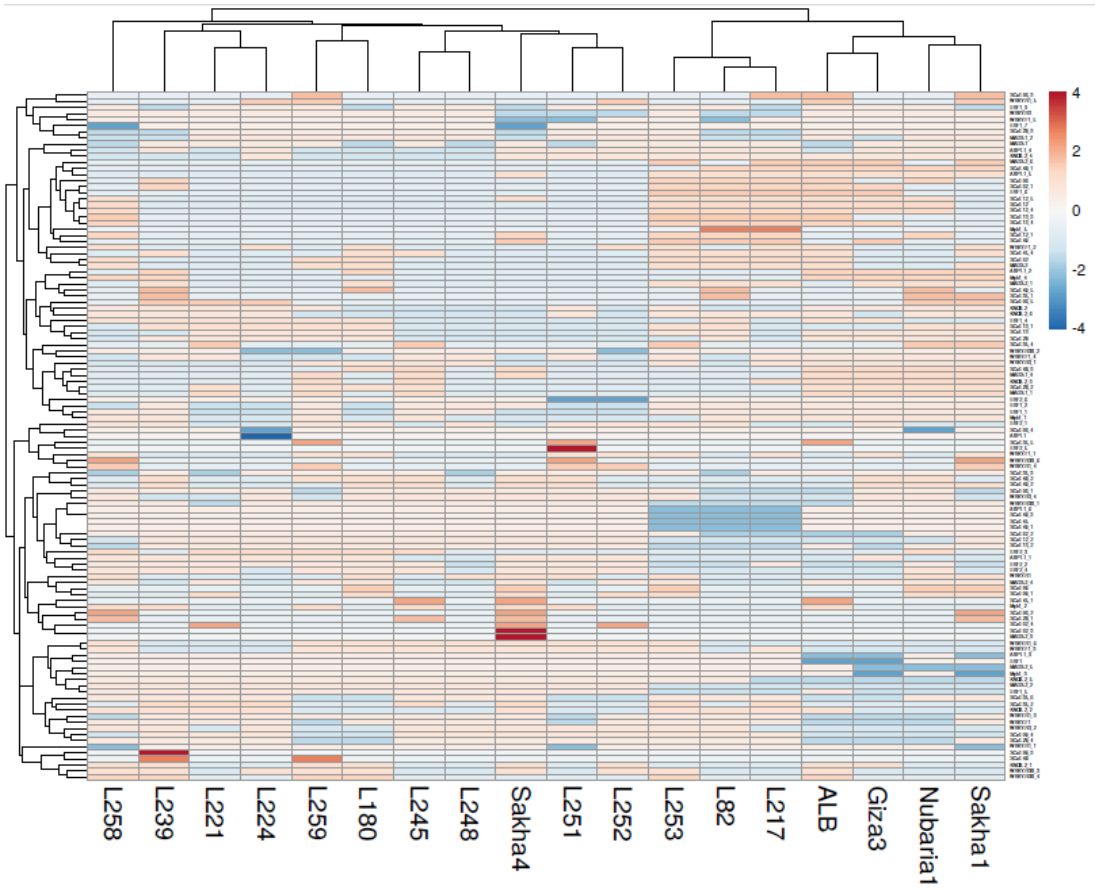

Fig. 2. Multivariate heatmap illustrating the genetic diversity of 18 faba bean genotypes based on polymorphisms of SCoTs and CDDP markers; the module heatmap of ClustVis—an online tool for clustering and visualizing multivariate data—was used (Metsalu *et al*., 2015).

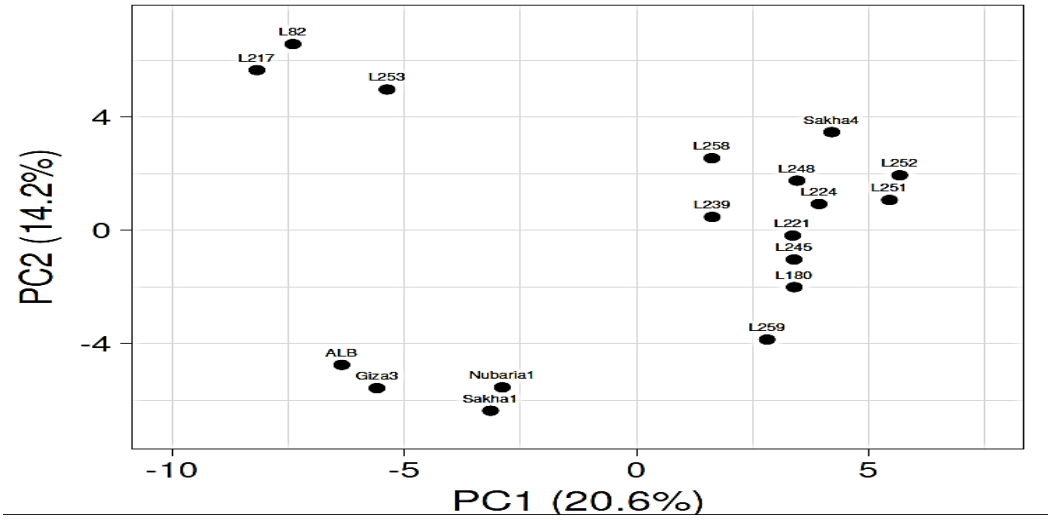

Fig. 3. Illustration of the genetic diversity of 18 faba bean genotypes according to principal component analysis (PCA) based on polymorphisms of SCoTs and CDDP markers using PAST software.

Combined dendrogram and similarity indices

Based on the data from 21 of the 50 SCoT and CDDP markers for the studied faba bean accessions and cultivars, a genetic distance tree was constructed using Dice's genetic similarity matrix (Figure 4). In this tree, L259, L180, L221, L224 and L239 were close to the other accessions, while the L258 accession was located alone in the tree. In Egypt, these accessions are located nearby. Moreover, Sakha 4, L245, L248, and L251 were found together in the second cluster of the tree, and the generic distance for a faba bean breeder was explored. On the other hand, L253, L217, L82, ALB, Giza 3, Nubria 1 and Sakha 1 were segregated into two groups in the third cluster. All the accessions and cultivars were distributed in three clusters.

According to the SCoTs and CDDP molecular marker polymorphisms, a similarity matrix was constructed among the 18 accessions and cultivars based on Dice coefficient (Table 5). According to the similarity matrix of SCoTs and CDDP analysis, the highest similarity value (85%) was observed between L224 and L221. Conversely, the lowest similarity value (52%) was observed between L82 and L251, indicating that these accessions and cultivars were distantly related, as shown in Table 1, Table 2 and Figure 3. These results are nearly consistent with those of previous studies (Elshafei *et al*., 2019 and Amer *et al*., 2014).

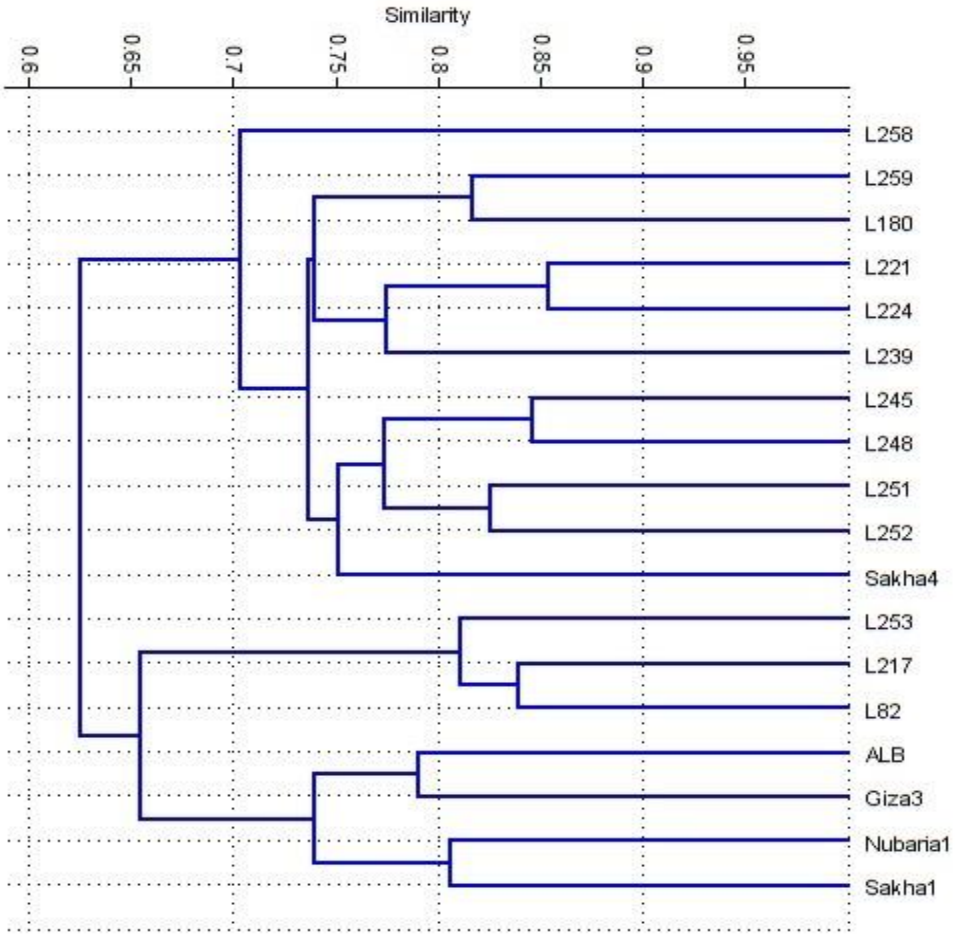

Fig. 4. Cluster tree of genetic distance between 18 faba bean accessions and cultivars based on the analysis of 21 SCoTs and CDDP primers according to Euclidean distance and the UPGMA algorithm in PAST software.

Table 5. Genetic similarity of the 18 faba bean accessions and cultivars based on SCoTs and CDDP markers.

	L82	L217	L221	L224	L239	L245	L248	L251	L252	Sakha4	ALB	Giza3	Nubaria1	Sakha1	L253	L258	L259	L180
L82	1.00																	
L217	0.84	1.00																
L221	0.60	0.57	1.00															
L224	0.61	0.57	0.85	1.00														
L239	0.68	0.64	0.77	0.78	1.00													
L245	0.56	0.62	0.79	0.74	0.76	1.00												
L248	0.60	0.59	0.77	0.76	0.72	0.84	1.00											
L251	0.52	0.52	0.70	0.73	0.71	0.75	0.79	1.00										
L252	0.54	0.56	0.76	0.81	0.70	0.75	0.80	0.82	1.00									
Sakha4	0.58	0.60	0.69	0.70	0.73	0.78	0.72	0.73	0.78	1.00								
ALB	0.65	0.70	0.62	0.60	0.64	0.64	0.58	0.54	0.55	0.54	1.00							
Giza3	0.63	0.65	0.60	0.58	0.65	0.65	0.60	0.58	0.58	0.59	0.79	1.00						
Nubaria1	0.63	0.62	0.72	0.67	0.71	0.73	0.64	0.63	0.59	0.65	0.72	0.79	1.00					
Sakha1	0.58	0.64	0.68	0.64	0.67	0.70	0.62	0.60	0.61	0.61	0.72	0.73	0.80	1.00				
L253	0.79	0.82	0.67	0.68	0.67	0.66	0.70	0.62	0.67	0.67	0.70	0.67	0.70	0.67	1.00			
L258	0.63	0.62	0.67	0.68	0.70	0.68	0.70	0.72	0.71	0.71	0.65	0.60	0.62	0.59	0.71	1.00		
L259	0.55	0.55	0.74	0.73	0.73	0.77	0.70	0.71	0.76	0.65	0.73	0.68	0.68	0.68	0.63	0.72	1.00	
L180	0.55	0.54	0.75	0.73	0.75	0.75	0.70	0.71	0.78	0.72	0.65	0.65	0.71	0.69	0.69	0.72	0.82	1.00

Chemical composition

The chemical composition of the analyzed faba bean accessions was estimated (Table 6). Notably, the moisture content differed significantly between the varieties. The three samples with the highest moisture content were L217 (8.190%), L82 (8.113%), and L224 (7.807%). The two samples with the lowest moisture content were L245 (2.643 %), and Sakha 4 (2.673%). For Nubira 1, the protein content was 23.083% and for L245 it was 35.857%. The ash residue is often used to calculate a material's mineral composition. The highest ash contents were found in L224, V239, L245, A.L.B., L253, L258, and Sakha 1, while the lowest were found in Giza 3 and L251. The fat content of L245 was the greatest at 2.780%, while L221 was the lowest at 1.527 %. Nubria 1, L251, and L252 had the lowest crude fiber contents, while L224 had the highest (9.827%). The highest and lowest amounts of carbohydrates were found in Nubria 1 and L252, respectively, and L245.

Table 6. Chemical composition (%): moisture, protein, fat, crude fiber and total carbohydrates for 18 accessions from faba beans.

Accessions	Moisture	Ash	Protein	Fat	Crude fiber	Total carbohydrates
L82	8.113 ±0.021 b	3.750 ±0.02 c	24.173 ±0.015 g	2.237 ±0.025 b	5.137 ±0.025 h	56.590 ±0.089 c
L217	8.190 ±0.01 a	3.807 ±0.015 b	25.563 ±0.015 f	1.637 ±0.015 e	5.750 ±0.02 f	55.053 ±0.025 d
L221	7.610 ±0.02 d	3.693 ±0.015 de	31.667 ±0.153 d	1.527 ±0.025 f	6.457 ±0.025 e	49.047 ±0.133 h
L224	7.807 ±0.015 c	3.937 ±0.025 a	27.753 ±0.015 e	2.130 ±0.02 c	9.827 ±0.015 a	48.547 ±0.029 i
V 239	3.130 ±0.02 g	3.910 ±0.01 a	32.250 ±0.056 c	1.423 ±0.025 g	7.6330 ±0.031 b	51.653 ±0.135 g
L245	2.643 ±0.04 h	3.917 ±0.006 a	35.857 ±0.067 a	2.780 ±0.02 a	7.547 ±0.021 c	47.257 ±0.06 j
V 248	7.09±0.014 e	3.63 ±0.011 e	24.182 ±0.019 g	2.128 ±0.016 c	4.573 ±0.08 j	58.397±0.134 b
L251	7.103 ±0.011 e	3.542 ±0.011 f	24.096 ±0.11 g	2.131 ±0.012 c	4.550 ±0.011 j	58.578 ±0.054 b
L252	6.065 ±0.013 f	3.661 ±0.014 e	23.087 ±0.064 h	2.154 ±0.015 c	4.55 ±0.021 j	60.497 ±0.083 a
Sakha 4	2.673 ±0.031 h	3.707 ±0.012 cd	33.633 ±0.042 b	1.913 ±0.015 d	5.633 ±0.035 g	52.440 ±0.044 f
ALB	7.58 ±0.028 d	3.893 ±0.013 a	25.61 ±0.281 f	1.86 ±0.035 d	6.698 ±0.042 d	54.359 ±0.175 e
Giza 3	7.107 ±0.015 e	3.543 ±0.021 f	24.090 ±0.02 g	2.130 ±0.02 c	5.030 ±0.03 i	58.100 ±0.066 b
Nubaria 1	6.063 ±0.015 f	3.647 ±0.015 e	23.083 ±0.074 h	2.160 ±0.02 c	4.530 ±0.02 j	60.517 ±0.091 a
Sakha 1	7.623 ±0.025 d	3.923 ±0.015 a	25.567 ±0.252 f	1.857 ±0.049 d	6.790 ±0.036 d	54.240 ±0.276 e
L253	7.102 ±0.018 e	3.885 ±0.012 a	25.43 ±0.018 f	2.26 ±0.027 b	5.035 ±0.05 i	56.288 ±0.047 c
L258	3.16 ±0.015 g	3.921 ±0.02 a	32.30 ±0.082 c	1.667 ±0.018 e	5.690 ±0.012 f	53.362 ±0.053 f
V 259	5.98 ±0.018 f	3.65 ±0.013 e	31.584 ±0.162 d	2.30 ±0.021 b	5.14 ±0.018 h	51.346 ±0.124 g
V 180	3.191 ±0.018 g	3.81 ±0.011 b	32.261 ±0.072 c	1.682 ±0.013 e	6.421 ±0.018 e	52.635 ±0.032 f
p-value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

The clustering of genotypes based on the chemical composition traits were classified into seven main groups (clusters) (Tables 7, 8, fig. 5. The sixth cluster had one genotype (**L $_{245}$**) which had the highest ratio each to protein% & ash% and the lowest ratio each to moisture% & total carbohydrates%. The lowest fat% obtained from fifth cluster which had four genotypes (**L $_{239}$, Sakha $_4$, L $_{258}$, L $_{180}$**) and similarity level 81.38%. The lowest crude fibers% obtained from seventh cluster which had two genotypes (**L $_{252}$, Nubaria $_1$**) and similarity level 99.82%. The fifth cluster consisted of two genotypes (**L 245, L 248**) which had the earliest fruiting date and similarity level of 76.90% between two genotypes. The sixth cluster similarity level between its genotypes (**Sakha 4, Giza 3)** was 67.54% and which had the earliest fruiting date. The seventh cluster had one genotypes (**Nubaria1**), earliest first fruiting date, the highest pod length (cm) and 100-seed weight (g). Finally, the eighth cluster consisted of three genotypes (**Sakha 1, L 258, L253**) which had the highest plant length (cm) and 74.09% similarity level between its genotypes.

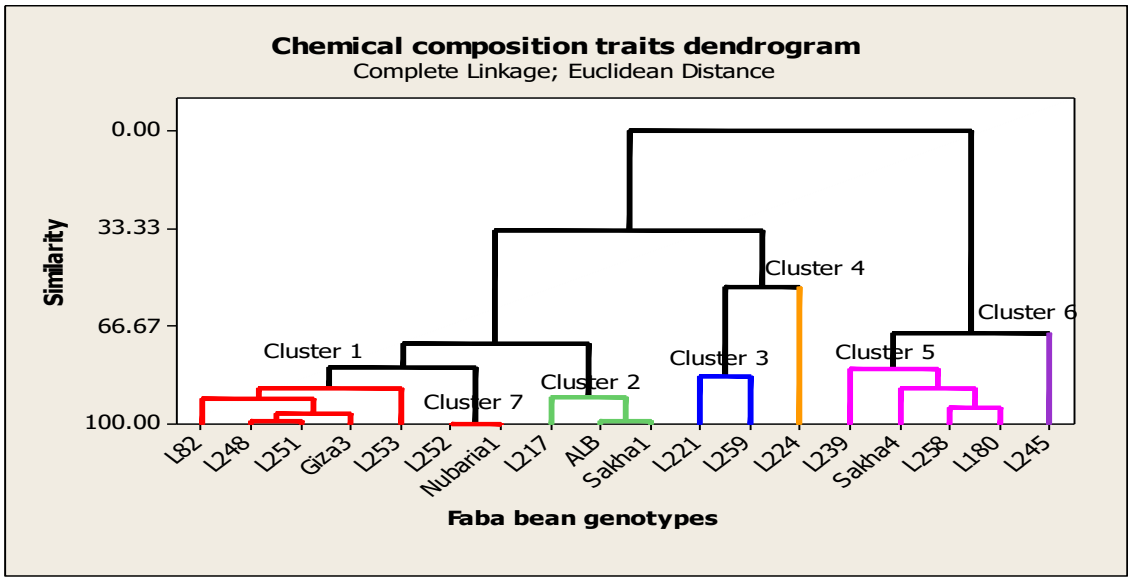

Fig. 5. Clustering of the eighteen faba bean genotypes using studied chemical composition traits.

Table 7. Cluster mean of the eighteen faba bean genotypes using studied chemical composition traits.

Cluster / Traits (%)	1	2	3	4	5	6	7
Moisture	7.30	7.80	6.80	7.81	3.04	2.64	6.06
Ash	3.67	3.87	3.67	3.94	3.84	3.92	3.65
Protein	24.39	25.58	31.63	27.75	32.61	35.86	32.09
Fat	2.18	1.78	1.91	2.13	1.67	2.78	2.16
Crude fibers	4.87	6.41	5.80	9.83	6.34	7.55	4.54
Total carbohydrates	57.59	54.55	50.20	48.55	52.52	47.26	60.51

Table 8. Cluster analysis summary showing the included genotypes and similarity level cluster of the 18 faba bean genotypes using studied chemical composition traits.

Cluster	Similarity level	Genotypes No.	Genotypes included
1	88.06	5	L 82, L 248, L 251, Giza 3, L 253
2	91.09	3	L 217, ALB, Sakha 1
3	83.54	2	L 221, L 259
4	53.38	1	L 224
5	81.38	4	L 239, Sakha 4, L 258, L 180
6	69.11	1	L 245
7	99.82	2	L 252, Nubaria 1

Biplot, Heatmap and Morphological Traits

A biplot was explored based on all the studied characteristics (Fig. 6 A). All of accessions and cultivars included in this study were divided into two groups. There were 22 primers for SCoTs and CDDP markers, chemical traits and field performance, but these were less common than the first and largest groups in the collection of faba bean accessions and cultivars. Moreover, this difference also appeared in the heatmap as the reason for the emergence of L82, L221, L217, ALB, L224, Sakha 1, L258, and L253 among the L259 accessions and cultivars. L239, L180, L245 and Sakha 4 accessions and cultivars are known for their traits during the period of faba bean breeds. The remaining accessions, L248, L252, Giza 3, L251 and Nubria 1, were located together according to the effectiveness of all the traits measured in this study. Furthermore, all the accessions are rare; they are characterized by a low content of tannin compounds, which makes them promising for use in breeding beans that do not cause anemia. Moreover, these genotypes explain the breed's concept of improving faba bean breeding by evaluating accessions with old and new cultivars.

Similarly, the biplot of the PCA-associated loading plot showed that, while SCoT and CDDP were used as markers, Ash %, crud fiber, pod no/node, protein %, pod length and the seed no/pod trait were positively correlated with L 239, L 245, L 180, L 258 and L 221, respectively; seed weight per plant; 100 seed weight; the end of the flowering date; the first flowering date; and fruiting date were associated with Nubria 1 cultivars. Furthermore, moisture %, total carbohydrates, seed no/pod plant height and fat % were more strongly associated with L248, L253, L252, L251, and L82. The remaining accessions and cultivars were related to the other chemical and morphological traits (Fig. 6 B and C).

The biplot and its associated heatmap explored the differences among the accessions and cultivars (Fig.

4) in balance with the PCA results. Briefly, the HCA-associated dendrogram based on SCoT and CDDP markers, chemical composition and field performance outputs showed that they were clustered separately into three distinct clusters. Furthermore, the HCA-associated dendrogram generated in this study showed that all the examined parameters were clustered into three distinct clusters. For further clarification, the clusters were distributed based on the SCOTs and CDDP markers, characteristics of the field performance, and chemical composition. Each cluster depends on the characteristics affected by it, as mentioned previously. Biplot could help to understand and analyze the results of the complex biological questions posed in the world of agriculture and the impact of genetic variation on plant performance and breeding. These results are similar to the data of (Mohamed *et al*., 2021; Rizk *et al*., 2023 and Omar *et al*., 2023).

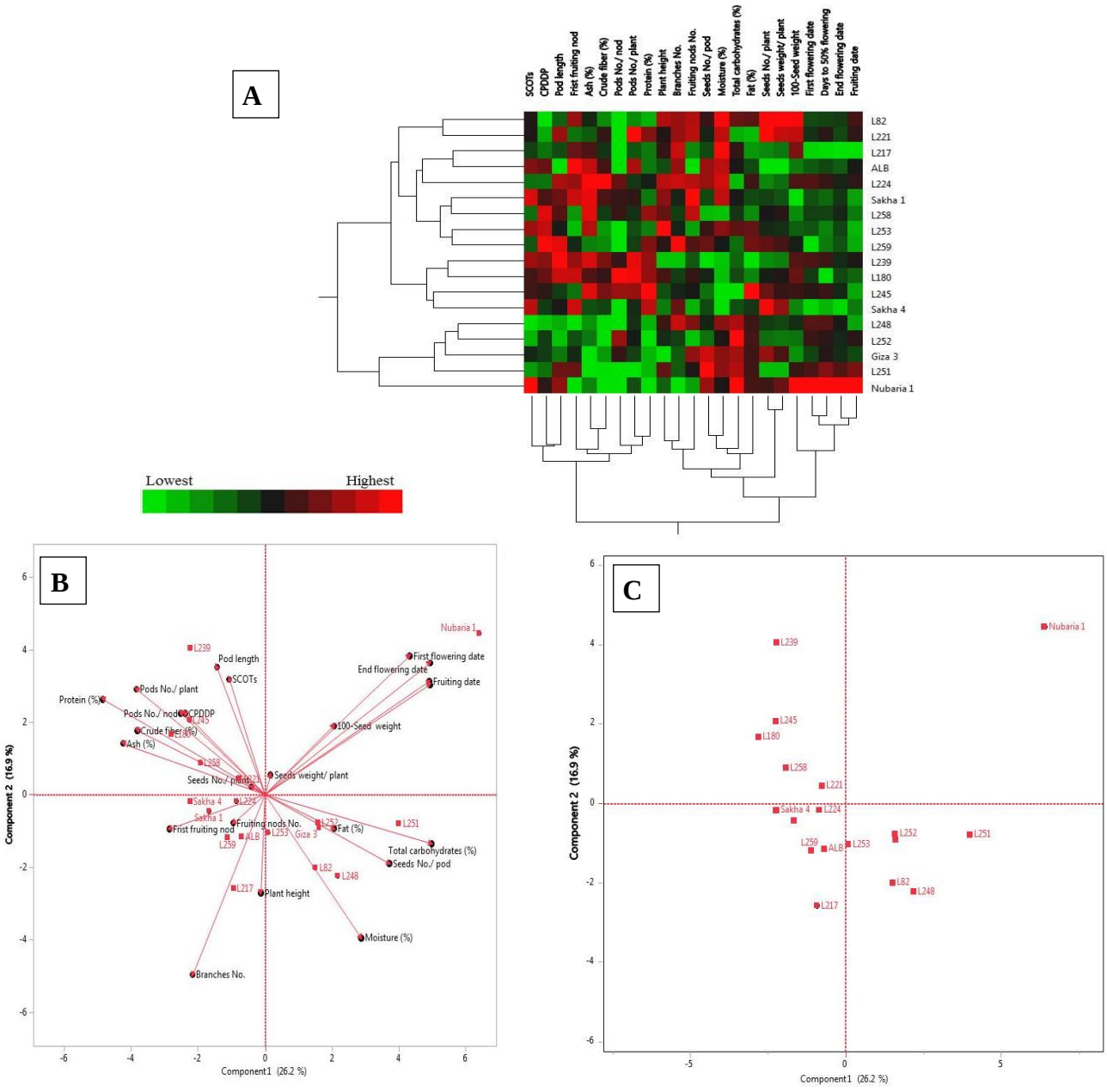

Fig. 6. A. Cluster analysis and heatmap based on SCoTs and CDDP markers chemical composition and morphological traits of eighteen faba bean accessions and cultivars. Heatmap was constructed using JMP®, Version 15 (SAS Institute, Inc., Cary, NC, 1989—2019). B and C biplot displaying a cluster tree illustrating the genetic distance based on the analysis of: proximate constituents, nutritive value, polymorphisms for 22 SCoTs and CDDP primers and morphological traits for the eighteen faba bean accessions and cultivars using Euclidean distance and the UPGMA algorithm in PAST software.

Field Traits

The cluster analysis was used as an efficient procedure to emerge the structural relationships among tested genotypes. Provides a hierarchical classification of them (Polignano *et al.*, 1989) obtained with the average linkage procedure; **UPGMA** (un-weighted pair group method using arithmetic average) developed by Sokal and Michener (1958). Genotypes were classified into eight main groups (clusters) using studied field traits where each group contained the genotypes that showed similar phenotypic performance (Tables 9&10, Figure 7).

The first cluster consisted of two genotypes (**L82, L221**) which had the highest branches No., fruiting nods No., seeds No. and seeds weight/ plant and 74.91% similarity level between its two genotypes. The second cluster consisted of three genotypes (**L217, L259, and L180**) which had the earliest first fruiting date, days to 50% flowering and fruiting date and 71.48% similarity level between its genotype. The third cluster consisted of two genotypes (**L224, L 251**) which had the highest seed No./ pod and similarity level 74.31% between two genotypes. The fourth cluster similarity level between its genotypes (**L 239, L 252, ALB)** was 62.73% and which had the highest seeds No. / plant.

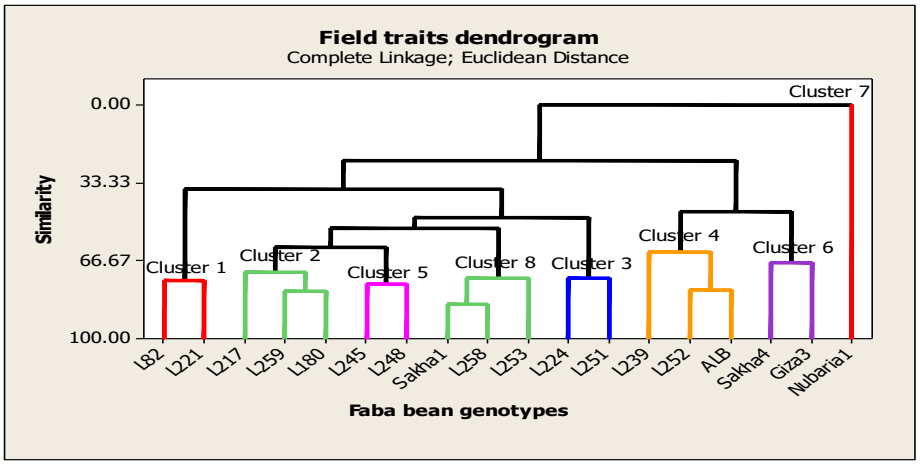

Fig. 7. Cluster analysis based on field traits Distribution of the eighteen faba bean genotypes.

Table 9. Cluster means of the eighteen faba bean genotypes using the studied field traits.

Traits \ Cluster	1	2	3	4	5	6	7	8
Plant height (cm)	108.44	105.21	110.94	95.42	101.88	89.06	96.25	112.29
Branches No.	3.25	3.21	3.00	2.71	3.13	2.88	2.00	2.83
First fruiting nod	3.69	3.88	3.94	3.92	3.19	3.88	3.00	3.50
Fruiting nods No.	10.31	7.46	9.09	4.90	8.59	7.22	5.06	9.00
Pods No./ nod	1.50	1.75	1.69	1.75	1.75	1.63	1.50	1.67
Pods No./ plant	15.94	15.88	13.5	17.00	16.13	15.06	14.63	15.21
Pod length (cm)	8.81	9.79	9.94	7.46	7.19	7.94	10.00	9.21
Seeds No./ bod	2.94	2.92	3.38	2.71	2.75	3.06	3.25	2.71
Seeds No./ plant	34.75	24.79	19.38	19.54	26.44	32.63	27.13	24.75
Seeds weight/ plant (g)	35.63	24.15	18.89	18.47	24.42	29.49	28.95	23.71
100-Seed weight (g)	106.33	100.08	99.44	95.12	97.76	91.41	107.76	90.70
First fruiting date	41.81	38.38	46.50	44.13	45.50	39.19	57.63	41.33
Days to 50% flowering	47.88	42.00	52.69	48.71	51.25	45.56	63.00	45.25
End flowering date	51.56	49.67	55.25	51.79	52.56	49.63	72.88	51.92
Fruiting date	67.69	61.88	69.81	66.08	61.88	63.63	81.75	62.17

Table 10. Cluster analysis summary showed the included genotypes and similarity level cluster of the eighteen faba bean genotypes using studied field traits.

Cluster	Similarity level	Genotypes No.	Genotypes included
1	74.91	2	L 82, L 221
2	71.48	3	L 217, L 259, L 180
3	74.31	2	L 224, L 251
4	62.73	3	L 239, L 252, ALB
5	76.90	2	L 245, L 248
6	67.54	2	Sakha 4, Giza 3
7	0	1	Nubaria 1
8	74.09	3	Sakha 1, L 258, L 253

Field and chemical composition traits cluster

Genotypes were classified into eight main groups (clusters) using studied field & chemical composition traits where each group contained the genotypes that showed similar phenotypic performance (Tables 11, 12 and Figure 8).

The first cluster consisted of two genotypes (**L82, L221**) which had the highest branches No., fruiting nods No., seeds No. and seeds weight/ plant and 70.53% similarity level between its two genotypes. The second cluster consisted of two genotypes (**L217, ALB**) which had the earliest first fruiting date, days to 50% flowering and end flowering date and 64.77% similarity level between its genotypes. The fourth cluster consisted of one genotype (**L239**) which had the highest pod length (cm) and ash% and the lowest ratio for each to moisture% and fat%.

The fifth cluster similarity level between its genotypes (**L 245, L 259, L 180)** was 69.89% and which had the highest pods No./ nod, pods No./ plant & protein% and the lowest total carbohydrate%. The lowest crude fibers% obtained from seventh cluster which had one genotype (**Nubaria 1**) and the highest seeds No./ pod & 100-sees weight (g) while early first fruiting nod. Finally, the eighth cluster consisted of three genotypes (**Sakha 1, L 258, L253**) which had the highest plant length (cm) & the earliest fruiting date and 69.87% similarity level between its genotype. These findings are in accordance with results obtained by Shadakshari *et al* (2011), Sheykhi *et al*. (2014) and Pallavi *et al* (2020) and they used similar technique.

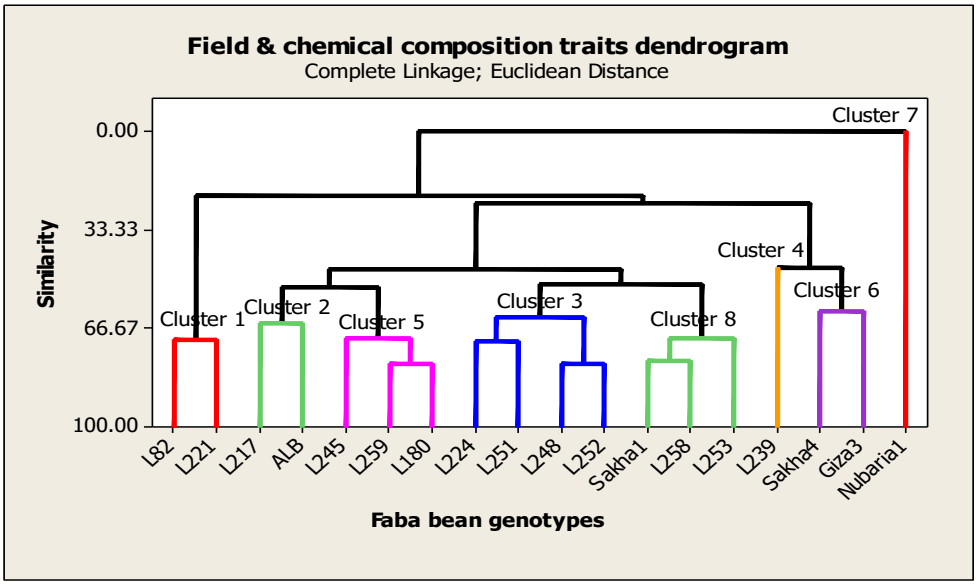

Fig. 8. Distribution of eighteen faba bean genotypes in seven clusters using studied field and chemical composition traits.

Table 11. Cluster analysis of the eighteen faba bean genotypes using studied field and chemical composition traits.

Cluster / Traits	1	2	3	4	5	6	7	8
Plant height	108.44	101.56	107.19	86.88	102.92	89.06	96.25	112.29
Branches No.	3.25	3.31	3.03	2.13	3.04	2.88	2	2.83
Frist fruiting nod	3.69	4.06	3.59	4	3.71	3.88	3	3.5
Fruiting nods No.	10.31	4.66	8	6.5	8.21	7.22	5.06	9
Pods No./ nod	1.5	1.5	1.72	1.75	1.92	1.63	1.5	1.67
Pods No./ plant	15.94	15.94	14.41	18.13	16.75	15.06	14.63	15.21
Pod length	8.81	6.63	8	11.13	9.88	7.94	10	9.21
Seeds No./ pod	2.94	3	3.06	2.25	2.88	3.06	3.25	2.71
Seeds No./ plant	34.75	18.56	21.13	20.38	27.54	32.63	27.13	24.75
Seeds weight/ plant	35.63	17.79	20.14	19.91	26.15	29.49	28.95	23.70
100-Seed weight	106.33	97.13	97.01	100.33	99.13	91.41	107.76	90.70
First flowering date	41.81	38.88	46.28	46.25	40.79	39.19	57.63	41.33
Days to 50% flowering	47.88	43.61	51.69	50.5	45.17	45.56	63	45.25
End flowering date	51.56	48.69	53.91	52.25	51.58	49.63	72.88	51.92
Fruiting date	67.69	62.88	67.06	66.13	62.38	63.63	81.75	62.17
Moisture (%)	7.86	7.89	7.016	3.13	3.94	4.89	6.06	5.96
Ash (%)	3.72	3.85	3.69	3.910	3.79	3.61	3.65	3.91
Protein (%)	27.92	25.59	24.78	32.25	33.23	28.86	23.083	27.77
Fat (%)	1.88	1.75	2.14	1.42	2.25	2.02	2.16	1.91
Crude fiber (%)	5.8	6.22	5.88	7.63	6.37	5.33	4.53	5.84
Total carbohydrates (%)	52.82	54.71	56.51	51.65	50.41	55.27	60.52	54.63

Table 12. Cluster analysis summary showed the included genotypes and similarity level cluster of the 18 faba bean genotypes using studied field & chemical composition traits.

Cluster	Similarity level	Genotypes No.	Genotypes included
1	70.53	2	L 82, L 221
2	64.77	2	L 217, ALB
3	62.96	4	L 224, L 251, L 248, L 252
4	46.30	1	L 239
5	69.89	3	L 245, L 259, L 180
6	61.17	2	Sakha 4, Giza 3
7	0	1	Nubaria 1
8	69.87	3	Sakha 1, L 258, L 253

Conclusion

In the present study, the similarity levels of the 18 faba bean genotypes were estimated on the basis of molecular genetics, field traits and chemical composition traits. This investigation will be effective to assess the extent of available variability, which will be useful for selecting superior genotypes on the basis of their phenotypic expression so as to use them in breeding programs to improve the important traits.

Ethics approval and consent to participate

Not applicable

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