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RESEARCH ARTICLE

Unveiling genetic richness: Profiling broad bean diversity in the Nilgiri Hills through morphological, biochemical and SSR markers

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Abstract

This study aimed to uncover the genetic relationship among broad bean genotypes to establish a high-yielding strain to elevate the status of this underutilized vegetable in the country. Twenty broad bean genotypes were assessed using randomized block design to reveal their genetic connections derived from twelve morphological factors and seven biochemical attributes. In addition, SSR primers were used to examine the molecular differences. The multifaceted data obtained were combined to evaluate genetic distinctions among these genotypes. The genetic analysis revealed that pod production per plant exhibited superior values of both phenotypic and genotypic coefficient of variation. Association analysis of green pod yield featured a strong positive correlation with seed yield, carbohydrate content, pod count per plant and 100 seed mass. The most notable positive direct influence on green pod yield was due to 100 seed weight. Choosing traits with a strong correlation with pod yield, along with moderate to high levels of phenotypic coefficient of variation (PCV), genotypic coefficient of variance (GCV), heritability and genetic progress would boost the effectiveness of the broad bean improvement program. The cluster analysis sorted the 20 genotypes into six groups, highlighting the considerable variation within each group. Among the SSR primers screened, the peak PIC score of 0.660 was noticed for the primer GBSSR-VF-172. The dendrogram constructed based on SSR markers resulted in two major clusters, illustrating the genetic affiliations and diversity within the genetic lines. This multidimensional characterization highlighted significant genetic disparities among broad bean genotypes, facilitating the selection of superior genotypes to develop high-yielding cultivars.

Keywords

broad bean; correlation; genetic variability; path analysis; pod yield; SSR marker

Introduction

The broad bean (*Vicia faba* L.), commonly known as faba bean is an ancient, nutrition-rich leguminous crop that thrives in cool climates. It is cultivated worldwide and holds significant importance as a minor legume crop in India. Its significance in global agriculture is substantial, owing to its

superior yield potential compared to other grain legumes. The major producers of broad bean include Mediterranean countries, Ethiopia, China and Egypt (1). The seeds of broad beans can be consumed in various ways including dry, roasted, soaked, cooked, frozen or canned. The widespread consumption of this crop is hindered by the existence of specific antinutritional compounds which can lead to haemolytic anaemia known as favism. Their regular intake in substantial quantities as part of their diet can result in noticeable and advantageous long-term health effects (2).

Despite being an underutilised crop and grown in limited scale in India, broad bean fetches a good price in the Nilgiris district of Tamil Nadu. Since broad bean cultivation is not done commercially in the state, dearth of high yielding cultivars with good-quality pods is a primary constraint for growers in this region. To improve the pod yield through selection and breeding, it is crucial to access the genetic diversity hidden within the germplasm, whether at the morphological, biochemical or DNA level. The span of genetic diversity within the breeding material increases genetic improvement in the crops (3). Quantifying genetic diversity in genotypes, along with heritability assessments for yield and related traits, is key to targeted selection strategies (4). The outcome of yield is multifactorial trait, driven by connected traits. Correlation coefficients help to find these associations, but they can be misleading because the component traits are often interdependent and affect yield indirectly. To overcome this, path analysis can be done, which breaks down the correlation coefficient into direct and indirect effects, aiding in unravelling the association between yield and key traits.

Since morphological and biochemical markers are highly influenced by environment, the use of molecular markers is fundamental in evaluating genetic differences across a population. Among the DNA markers used for genotyping, SSRs or microsatellites stand out as dependable, precise, co-dominant, exceptionally polymorphic and cost-efficient options (5). Considering this, this study was undertaken to quantify genetic differences via morpho-biochemical traits and SSRs followed by path analysis for pod yield traits in broad bean genotypes to support the future breeding programs. This would contribute to the ultimate goal of finding a wide range of parent lines for creating populations with different traits and associating these features with molecular markers.

Materials and Methods

The research materials encompassed twenty distinct broad bean genotypes sourced from multiple locations of the Nilgiris district, Tamil Nadu, India focusing on plants that were high-yielding, disease-resistant and exhibited desirable morphological traits (Table 1). The genotypes collected were maintained at Woodhouse farm of Horticultural Research Station, TNAU, Udhagamandalam, Nilgiris, Tamil Nadu, India, where the field study was conducted during Kharif 2023. The farm is positioned at 2535 m above the MSL and is located at the geographical coordinates of approximately 11°25′28′′ North latitude and 76°43′22′′ East longitude. The crop was spaced 45×15 cm apart in a randomized block design (RBD) with three replicates. Standard agricultural practices were abided to raise a good crop.

Table 1. Name and geographical sources of broad bean genotypes

S. No.	Genotype	Source	Latitude	Longitude	Elevation
1	Vf 1	Melkowhatty	11°23′08′′N	76°39′29′′E	2,167 m
2	Vf 2	Hosahatty	11°17′50′′N	76°42′24′′E	1,837 m
3	Vf 3	Kadanad	11°31′59′′N	76°45′54′′E	918 m
4	Vf 4	Selatha	11°28′20′′N	76°43′55′′E	1,800 m
5	Vf 5	Sholur-Thattaneri	11°20′42′′N	76°37′54′′E	2,023 m
6	Vf 6	Sholur-Bikkaikandy	11°27′53′′N	76°41′09′′E	1,844 m
7	Vf 7	Kuruthukuli	11°23′20′′N	76°38′35′′E	2,192 m
8	Vf 8	Woodhouse farm	11°25′28′′N	76°43′22′′E	2,535 m
9	Vf 9	Bygamund	11°22′08′′N	76°40′31′′E	2,070 m
10	Vf 10	Kilkowhatty	11°22′45′′N	76°39′49′′E	2,077 m
11	Vf 11	Bengalmattam	11°17′25′′N	76°40′38′′E	1,847 m
12	Vf 12	Achanakal	11°22′52′′N	76°43′21′′E	2,060 m
13	Vf 13	Kilkowhatty ADA	11°22′44′′N	76°39′54′′E	2,072 m
14	Vf 14	Kookalthorai	11°29′24′′N	76°49′39′′E	1,522 m
15	Vf 15	Iduhatty	11°27′38′′N	76°46′41′′E	1,972 m
16	Vf 16	Thuneri	11°27′13′′N	76°44′06′′E	1,880 m
17	Vf 17	Kotagiri	11°25′25″N	76°52′17′′E	1,949 m
18	Vf 18	Conoor	11°21′45′′N	76°46′23′′E	1,906 m
19	Vf 19	Kenthorai	11°26′34″N	76°44′59′′E	2,101 m
20	Vf 20	Kundah	11°15′40″N	76°38′03′′E	1,851 m

Morphological and biochemical analysis

Each replication of each genotypes had five randomly chosen plant for observation of 12 morphological features namely plant height (cm), branch density, days to 50% flowering, maturity period, pod length (cm), pod width (cm), pod count per cluster, pod count per plant, seed count per pod, 100 seed weight (g), seed yield (g) and pod yield (g). The biochemical attributes such as moisture (%), dry matter (%), ascorbic acid (mg/100g), protein (%), phenol (%), carbohydrate (%) and total soluble solids (° brix) were also estimated according to AOAC Official Method (6). Descriptive statistics were analysed using the mean values for each characteristic.

The data were analysed using variance analysis to uncover key insights, in line with previous research (7). Subsequently, various biometric procedures were employed to calculate the genotypic coefficient of variance (GCV), phenotypic coefficient of variation (PCV) and heritability in a broad sense, genetic advance, correlation and path coefficient analysis (8-10). These genetic parameters were derived using OPSTAT software (11). The R-based web tool GRAPES, was leveraged to analyse correlation coefficients (12). Agglomerative cluster analysis was performed using statistical software for agricultural research (13).

Molecular Analysis

***Genomic DNA isolation and purification*:** Molecular studies were executed in the Molecular laboratory of Department of Vegetable Science, HC and RI, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu. Genomic DNA extraction for molecular analysis was done following the CTAB method (14). Leaf portion of selected genotypes (10 days old seedlings) raised in portrays were taken for DNA extraction. The DNA was further purified by RNase treatment. The quantity of extracted DNA was assessed using the Nano Drop™ 1000 from Nano Drop Technologies, USA. Using the quantification data, the DNA was diluted with 1X TE buffer to achieve a final level of 50ng/µl.

***SSR amplification*:** Sixteen SSR oligonucleotides from published literatures were incorporated in this investigation (15-17). The primers are listed in Table 2. PCR amplifications were performed using reaction mixture (10 µl) consisting of 2X master mix (5.0 µl), primer pairs (0.5 µl each), sterile double distilled water (3µl) and template DNA (1 µl). DNA amplification was performed using a Bio-Rad Eppendorf thermocycler. PCR conditions included an initial thermal denaturation of 8 min at 94℃, then 38 cycles of denaturation for 1 min at 94℃, annealing at 50-60℃, and followed by elongation for 2 min at 72℃. The final extension step was maintained at 72℃ for 7 minutes. PCR products were analysed on 3% agarose gel tinged with ethidium bromide. The amplified products were reproduced using a UV gel analyzer.

Molecular data analysis: The allelic data were converted into binary format, with "1" indicating presence of allele and "0" indicating its absence for genetic distance analysis. Polymorphism information content was computed as described in previous studies (18). The summary statistics namely number of alleles (Na), observed heterozygosity (Ho), expected heterozygosity (He), Shannon index (I) was computed using GenALEx 6.5 (19). The genetic similarity was assessed using Jaccard's coefficient of similarity based on this binary matrix, and a dendrogram was created using DARwin 6.0 software from these binary matrices.

Results and Discussion

The variance analysis for all characteristics exposed pronounced disparities across the genotypes, demonstrating the presence of greater diversity within them (Table 3). The existence of genetic diversity provides an oppurtunity to enhance the yield and related traits of broad beans through selection. Similar notable variations in all traits of broad bean have been reported in earlier studies (20-21).

Genetic variability studies

Genetic parameters related to pod yield and its components were studied to evaluate the impact of selection on different traits (Table 4). The degree of diversity among genotypes was evident from the substantial mean and range values observed for the variables examined. This study demonstrated the largest genotypic and phenotypic variances for total soluble solids subsequent to pod count per plant, branching frequency, seed yield, cluster pod count and protein, while weakest genotypic and phenotypic variances recorded for moisture followed by pod width, phenol and maturity and flowering period.

Table 2. List of SSR Primers used in the study

S. No.	SSR Primer (loci)	Sequences (5' - 3')	Annealing Temperature (°C)
1.	GBSSR-VF-172	F: CGGTTTCTAAATCTGGCG R: GCTCCATTGAAACCAATTCT	57
2.	GBSSR-VF-175	F: TGCCATTCCATCTGAACC R: CCAGGCAATGGAATCTGA	58
3.	GBSSR-VF-131	F: CCGTACTAAATGAAGCCTTT R: GGCAATCAAGTCCGGTAA	57
4.	GBSSR-VF-168	F: TCTCCAAACCCTCCTCGT R: TCAGCCACAAAATCAGCA	57
5.	GBSSR-VF-119	F: GTGGCCTGTACTGGTGGA R: ACTCGTTGGGGCTAGGAA	58
6.	GBSSR-VF-115	F: TGCTGCTTTTCCAACCAT R: GTGCATGCCATAACAAAA	57
7.	GBSSR-VF-113	F: TGGTGGTGCTTCTTTCCA R: TGGTGAGCTTGGAACTGC	59
8.	GBSSR-VF-28	F: AGAGTCCCAAAGAGTGGGTT R: CCAAAGGCAAAAATGAGGGCTT	52.5
9.	GBSSR-VF-20	F: TCCACCAAGTCCACCTGA R: AATAAGGGCGCAGGAGAG	50.8
10.	VfG 19	F: AGCGATGGTGCTCATGCTTA R: TCTCTCACGGAATCACATCTTT	50
11.	VfG 1	F: TTTCAGCAAACTAGAACCAATC R: GGCATTCAGTTTTTACCTTGTA	50
12.	VfG 44	F: GATGTTGTTGGTGTTGTTTA R: CAATTAGGAGCAAAATCAGA	50
13.	VfG 9	F: GGTTTTGAATAGAAATGCAA R: AAGATGTGTCAATATTGTTTT	50
14.	VfG 41	F: AGCCCATGGTTCAAATGCAA R: GCAGTCATGCCACTGCTTA	50
15.	CAAS5	F: TACATCAGTCCCGCAAATCA R: CCATGTAGCCGATTCCACTT	55
16.	CAAS7	F: GACCCAAGCCTTCACCACTA R: TGTGTGGGATCCATTTTGAA	60

The PCV estimates surpassed the GCV estimates significantly for all the traits. This minor disparity in magnitude suggests that environmental influences showed minimal effect on the manifestation of these traits, indicating that relatively simple selection methods can be employed to improve these characteristics. The higher extends of PCV and GCV werenoticed for pod count and pod yield, both exceeding 20%. These traits with greater PCV and GCV assessments point toward a significant level of genetic variability within them, offering ample opportunities for selection from different populations of broad bean to enhance production. These outcomes support those of prevoius studies in broad bean (20). The traits such as cluster pod count, seed count per pod, 100 seed weight, ascorbic acid, seed yield, phenol and protein, exhibited a moderate level of GCV and PCV. Prioritizing these traits during selection could lead to uncertainty in the improvement process. Reduced PCV and GCV approximations noticed for attributes like branch count, maturity and flowering time, pod length, pod width, moisture, dry matter, carbohydrate and total soluble solids. These observations are in agreement with previous works (21, 22). The low GCV and PCV values detected for these traits indicate that their potential response to selection would be lower compared to other traits.

The heritability was observed to be highest for the majority of attributes analyzed; indicating that much of variation was attributable to genetic factors. Substantial heritability (>60 %) along with substantial genetic advances, were obseved for branch count, pod count per cluster, pod count per plant, seed load per pod, ascorbic acid, 100 seed weight, protein, seed yield, phenol, carbohydrate content and pod yield. The presence of significant heritability and genetic advancement is explained by the impact of additive genetic effects, making these characteristics well-suited for phenotype-driven selection for their improvement (20).

Substantial heritability and modest genetic advancement were perceived for pod length, moisture content and plant height as recorded in previous studies (21). Significant heritability and minimal genetic advancement were witnessed in 50% flowering time, maturity period, pod width, dry matter and total soluble solids. This outcome can be attributed to non-additive genetic effects, suggesting that pursuing these traits through selection may not yield significant improvements, despite their apparent high heritability and limited genetic advance (22, 23).

Correlation coefficient analysis

Yield is an intricate trait that results from the interaction of multiple component traits and their interactions. Hence focusing exclusively on pod yield alone may produce misleading result and cause uncertainty. Correlation analysis plays a crucial role in examining the interrelationship and relative contribution of individual characteristics to the improvement of crops.

The pod yield showcased positive and markedly correlated with seed yield (0.90), 100 seed weight (0.83), pod count per plant (0.83), carbohydrate (0.82), pod width (0.77), seed count per bean (0.70), branch count (0.70), plant height

Table 3. Analysis of variance for various morphological and biochemical traits

Traits	Mean ± SE(m)	Range	CD at 5%	CV (%)
Plant height (cm)	95.27 ± 2.40	72.67 - 113.67	3.57	7.11
Branch density	2.60 ± 0.20	1.90 - 3.70	10.89	0.59
Days to 50% flowering	58.24 ± 0.49	52.71 - 63.97	1.19	1.46
Maturity period	156.18 ± 0.78	147.00 - 163.50	0.71	2.31
Pod length (cm)	11.01 ± 0.03	9.48 - 12.85	0.41	0.10
Pod width (cm)	1.58 ± 0.01	1.49 - 1.76	0.48	0.02
Pod count per cluster	2.43 ± 0.18	1.65 - 3.35	10.22	0.52
Pod count per plant	17.25 ± 0.64	11.17 - 29.00	5.24	1.89
Seed count per pod	2.58 ± 0.10	1.88 - 3.25	5.50	0.30
100 seed weight (g)	109.78 ± 0.91	79.50 - 139.25	1.18	2.70
Seed yield (g)	64.73 ± 0.56	45.00 - 81.50	1.23	1.67
Pod yield (g)	160.00 ± 3.39	51.67 - 273.67	3.25	10.87
Moisture (%)	12.93 ± 0.20	11.25 - 14.50	2.16	0.58
Dry matter (%)	87.08 ± 0.20	85.50 - 88.75	0.32	0.58
Ascorbic acid (mg/100g)	16.42 ± 0.26	13.41 - 19.45	2.27	0.78
Protein (%)	21.53 ± 0.34	15.64 - 26.13	2.22	1.00
Phenol (%)	0.16 ± 0.01	0.12 - 0.20	7.83	0.03
Carbohydrate (%)	48.60 ± 0.43	43.90 - 52.77	1.25	1.27
Total soluble solids (°Brix)	7.76 ± 0.13	6.93 - 8.26	2.38	0.39

Table 4. Components of genetic parameters for various traits in broad bean genotypes

Characters	σ^2g	σ^2p	GCV (%)	PCV (%)	H^2b (%)	GA	GAM (%)
Plant height (cm)	8.49	12.45	10.60	11.73	81.67	18.81	19.74
Branch density	0.48	0.56	18.38	21.36	74.00	0.85	32.57
Days to 50% flowering	2.83	3.07	4.86	5.27	84.92	5.37	9.22
Maturity period	3.91	4.30	2.50	2.75	82.61	7.32	4.68
Pod length (cm)	0.82	0.97	7.46	8.78	72.19	1.44	13.05
Pod width (cm)	0.07	0.08	4.29	5.01	73.30	0.12	7.56
Pod count per cluster	0.33	0.38	16.20	18.67	75.26	0.59	28.95
Pod count per plant	4.30	4.67	27.02	29.34	84.81	8.16	51.26
Seed count per pod	0.33	0.38	12.70	14.92	72.47	0.57	22.28
100 seed weight (g)	13.93	14.94	12.61	13.53	86.97	26.76	24.23
Seed yield (g)	10.88	11.66	17.08	18.3	87.04	20.91	32.82
Pod yield (g)	53.43	56.73	34.12	36.23	88.71	103.67	66.21
Moisture (%)	1.07	1.11	8.31	8.58	93.69	2.14	16.56
Dry matter (%)	1.07	1.11	1.22	1.24	97.59	2.17	2.49
Ascorbic acid (mg/100g)	1.69	1.73	10.29	10.53	95.36	3.40	20.69
Protein (%)	2.58	2.62	11.97	12.18	96.68	5.22	24.25
Phenol (%)	0.02	0.03	14.54	16.52	77.52	0.04	26.38
Carbohydrate (%)	2.58	2.65	5.31	5.45	94.78	5.17	10.65
Total soluble solids (°Brix)	0.42	0.46	5.46	5.95	84.02	0.80	10.30

σ^2g- Genotypic variance, σ^2p- Phenotypic variance, GCV- Genotypic coefficient of variation, PCV- Phenotypic coefficient of variation, H^2b- Broad sense heritability, GA- Genetic advance, GAM- Genetic advance as per cent of mean

(0.66), total soluble solids (0.64), pod count per cluster (0.55), dry matter (0.50), protein (0.44) and ascorbic acid (0.40) (Fig. 1). Positive and significant correlations among these traits indicated a high degree of heritability for each trait. This suggests that any positive enhancement in these characteristics could drive a rise in output of green pods. These findings align with those of previous investigations (20, 24). The pod yield had a significant negative affinity with maturity time (-0.77), phenol (-0.63), time to 50% bloom (-0.57) and moisture (-0.50). This substantial negative relationship between yield and these factors validate of previous research (21).

The inter correlation among important yield and component characters revealed positive significant dependence for maturity time (0.66) with moisture, pod width (0.78) with weight of 100 seeds, carbohydrate (0.87), seed count per pod (0.80), branch density (0.72) and total soluble solids (0.66) with seed yield, dry matter (0.84) with protein and ascorbic acid (0.43) with dry matter. These outcomes are in line with past studies (20).

The association analysis findings suggest that focusing on traits such as seed output, 100 seed weight, pod count, pod width, carbohydrate, plant height, seed load per pod, branching frequency, dry matter and cluster pod count may lead to simultaneous enhancement of pod output per plant. Since these traits are interconnected, selecting for any one of them could lead to improvements in the other traits, ultimately resulting in increased crop yield.

Path Coefficient Analysis

While correlation coefficients are useful for understanding complex traits such as pod yield, they adequately not revealed the influence of direct and indirect impacts of traits. In such cases, the path coefficient enables the breakdown of the correlation coefficient into direct and indirect effects of independent variables on the outcome. This analysis helps in identifying the specific component traits that can be considered for improving yield.

The path analysis revealed substantial positive direct influence of green pod yield with 100 seed mass (0.396), branching frequency (0.365), total soluble solids (0.322) and dry matter (0.316). Seed yield (0.279), pod length (0.220) and pod count per plant (0.205) recorded moderate positive direct effect on pod yield, whereas the seed count per pod (0.024) has registered a trivial positive effect (Table 5). These outcomes align with previous studies (25, 26). These results indicated the significance of these traits in the selection of plants for pod yield. Focusing on these features in selection would enhance broad bean yield and the mentioned characteristics simultaneously. Detrimental direct effect on green pod production per plant was observed for maturation duration (-0.405), carbohydrate (-0.680), phenol (-0.039) and days taken for 50% flowering (-0.233). These findings corroborated the results of previous investigations (21, 27).

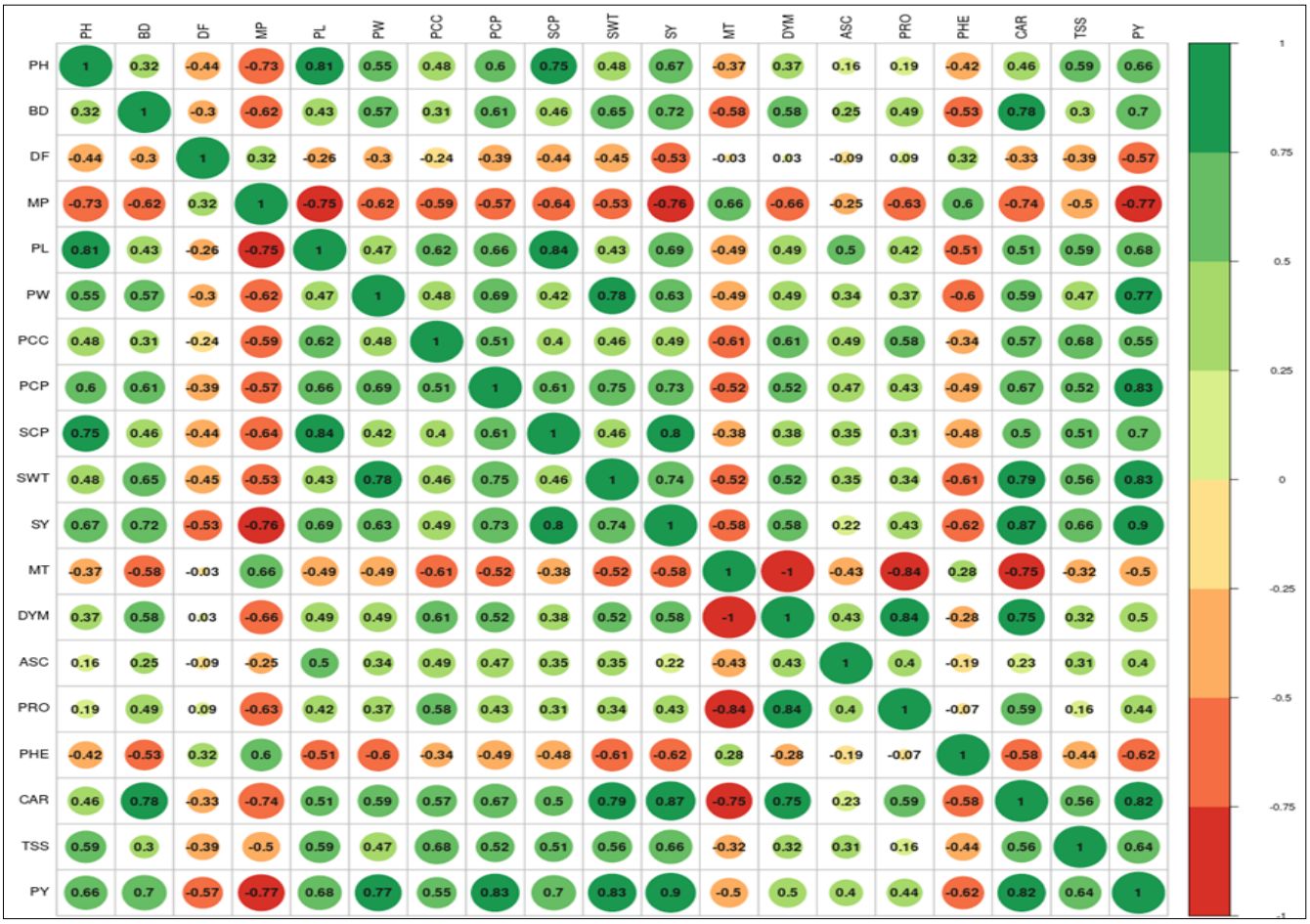

Fig. 1. Correlogram displaying the correlation coefficients of nineteen traits.

PH- Plant height (cm), BD- Branch density, DF- Days to 50% flowering, MP- Maturity period, PL- Pod length (cm), PW- Pod width (cm), PCC- Pod count per cluster, PCP- Pod count per plant, SCP- Seed count per pod, SWT- 100 seed weight (g), SY- Seed yield (g), MT- Moisture (%), DYM- Dry matter (%), ASC- Ascorbic acid (mg/100g), PRO- Protein (%), PHE- Phenol (%), CAR- Carbohydrate (%), TSS- Total soluble solids (°Brix), PY- Pod yield (g)

Plant height showed highest positive indirect influence on pod yield through maturity time coming after 100 seed weight, branch count, flowering duration and pod number per plant. This trait also recorded highest negative indirect effect *via,* carbohydrate. A high positive indirect impact on pod yield was exerted by pod width *via,* weight of 100 seeds, maturity time and branch density. Seed yield also recorded highest indirect effect on pod yield through maturity days, branch density and total soluble solids. The findings are in accordance with the literatures (20, 28). Regarding quality parameters, highest positive and negative indirect influence on green pod yield were observed with moisture content and dry matter respectively through carbohydrate.

The residual effect serves as a measure of how effectively the causal factor explains the variation in the dependent variable, which in this case is thepod yield. Here the residual value was determined to be 0.052. This suggests that all 19 traits studied were adequate for the genetic assessment of broad beans.

The path analysis outcomes showed that traits like 100 seed weight, branching frequency, seed yield, pod length, pod count, canopy height, total soluble solids and carbohydrate were the vital pod yield determinants, given their strong direct and indirect influence *via* many other characters.

Cluster analysis

The cluster analysis is a practical method for grouping germplasms and offers a solid basis for choosing core materials when planning breeding programs. The broad bean genotypes were segmented into six clusters based on with morphological and biochemical attributes, using the agglomerative cluster analysis method of hierarchical clustering with Euclidean distance as the similarity measure (Table 6) (Fig. 2). These six main clusters were labeled as clusters I to VI comprising of three, seven, four, three, one and two genotypes respectively. Cluster II represented the largest group with 7 genotypes, while cluster V had only one, making it the smallest cluster.

Table 5. Path coefficient analysis of 18 traits of broad bean

	PH	BD	DF	MP	PL	PW	PCC	PCP	SCP	SWT	SY	MT	DYM	ASC	PRO	PHE	CAR	TSS	PY
PH	**-0.233**	0.149	0.115	0.311	0.010	-0.083	-0.035	0.125	0.019	0.201	0.197	-0.033	0.135	-0.027	-0.009	0.018	-0.349	0.196	0.677*
BD	-0.095	**0.365**	0.075	0.255	-0.008	-0.091	-0.027	0.135	0.012	0.286	0.218	-0.052	0.208	-0.037	-0.021	0.023	-0.578	0.104	0.647*
DF	0.115	-0.118	**-0.233**	-0.145	0.066	0.043	0.021	-0.081	-0.011	-0.177	-0.150	0.002	-0.008	0.011	-0.004	-0.013	0.238	-0.131	-0.559*
MP	0.179	-0.230	-0.083	**-0.405**	-0.013	0.091	0.053	-0.118	-0.016	-0.204	-0.212	0.053	-0.214	0.028	0.026	-0.026	0.506	-0.179	-0.749*
PL	-0.011	-0.014	-0.069	0.023	**0.220**	-0.029	-0.015	0.038	0.000	0.006	-0.022	-0.011	0.044	-0.038	-0.013	-0.002	0.035	-0.014	0.129
PW	-0.133	0.229	0.069	0.254	0.044	**-0.145**	-0.039	0.144	0.010	0.313	0.182	-0.045	0.180	-0.043	-0.016	0.025	-0.413	0.162	0.763*
PCC	-0.113	0.135	0.067	0.296	0.045	-0.079	**-0.072**	0.098	0.010	0.171	0.132	-0.048	0.195	-0.051	-0.025	0.019	-0.385	0.183	0.561*
PCP	-0.143	0.241	0.092	0.233	0.041	-0.102	-0.034	**0.205**	0.016	0.300	0.205	-0.044	0.178	-0.060	-0.018	0.021	-0.469	0.174	0.816*
SCP	-0.187	0.191	0.106	0.274	-0.002	-0.064	-0.031	0.135	**0.024**	0.193	0.235	-0.033	0.133	-0.048	-0.013	0.020	-0.372	0.172	0.674*
SWT	-0.118	0.264	0.104	0.209	0.003	-0.115	-0.031	0.155	0.011	**0.396**	0.207	-0.046	0.185	-0.045	-0.014	0.025	-0.543	0.184	0.824*
SY	-0.164	0.286	0.125	0.307	-0.017	-0.095	-0.034	0.150	0.020	0.293	**0.279**	-0.048	0.192	-0.028	-0.018	0.027	-0.599	0.223	0.892*
MT	0.099	-0.240	-0.006	-0.274	-0.030	0.083	0.044	-0.115	-0.010	-0.232	-0.169	**0.079**	-0.316	0.064	0.036	-0.013	0.540	-0.111	-0.567*
DYM	-0.099	0.240	0.006	0.274	0.030	-0.083	-0.044	0.115	0.010	0.232	0.169	-0.079	**0.316**	-0.064	-0.036	0.013	-0.540	0.111	0.567*
ASC	-0.050	0.108	0.021	0.089	0.066	-0.050	-0.029	0.098	0.009	0.141	0.062	-0.040	0.161	**-0.126**	-0.017	0.008	-0.158	0.109	0.394
PRO	-0.053	0.187	-0.023	0.254	0.069	-0.056	-0.043	0.089	0.008	0.138	0.122	-0.068	0.273	-0.052	**-0.042**	0.002	-0.410	0.053	0.439*
PHE	0.106	-0.219	-0.080	-0.269	0.013	0.092	0.036	-0.109	-0.012	-0.253	-0.192	0.026	-0.103	0.026	0.003	**-0.039**	0.442	-0.133	-0.594*
CAR	-0.120	0.311	0.081	0.302	-0.011	-0.088	-0.041	0.141	0.013	0.317	0.246	-0.063	0.251	-0.029	-0.025	0.025	**-0.680**	0.198	0.806*
TSS	-0.142	0.118	0.095	0.225	-0.010	-0.073	-0.041	0.111	0.013	0.227	0.194	-0.027	0.109	-0.042	-0.007	0.016	-0.417	**0.322**	0.606*

Residual effect = 0.0522, Bold figures indicate the direct effects

PH- Plant height (cm), BD- Branch density, DF- Days to 50% flowering, MP- Maturity period, PL- Pod length (cm), PW- Pod width (cm), PCC- Pod count per cluster, PCP- Pod count per plant, SCP- Seed count per pod, SWT- 100 seed weight (g), SY- Seed yield (g), PY- Pod yield (g), MT- Moisture (%), DYM- Dry matter (%), ASC- Ascorbic acid (mg/100g), PRO- Protein (%), PHE- Phenol (%), CAR- Carbohydrate (%), TSS- Total soluble solids (°Brix)

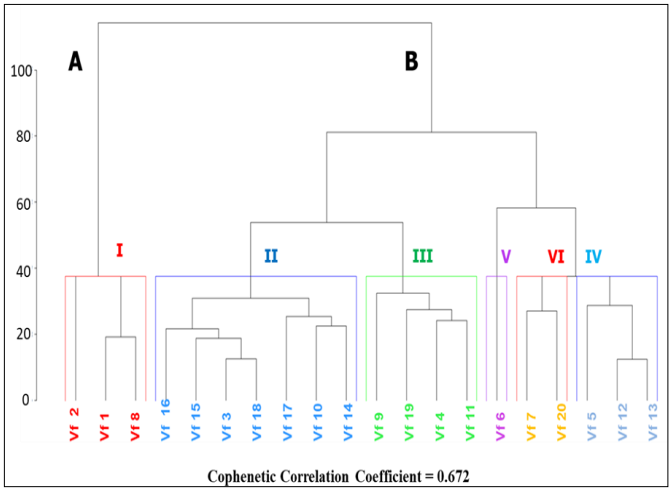

Fig. 2. Agglomerative cluster analysis of 20 genotypes of broad bean

Table 6. Hierarchical cluster grouping of Broad bean genotypes

Cluster	Cluster size	Genotypes under each cluster	Percentage of Genotypes
I	3	Vf 1, Vf 2 and Vf 8	15
II	7	Vf 3, Vf 10, Vf 14, Vf 15, Vf 16, Vf 17 and Vf 18	35
III	4	Vf 4, Vf 9, Vf 11 and Vf 19	20
IV	3	Vf 5, Vf 12 and Vf 13	15
V	1	Vf 6	5
VI	2	Vf 7 and Vf 20	10

The cluster diagram, derived from the mean data, highlighted three genotypes (Fig. 3), namely Vf 1, Vf 2 and Vf 8, which attained the highest pod and seed production per plant, which belonged to cluster I. In comparison, the genotypes in cluster V and VI yielded less. Genotypes within cluster II demonstrated moderate performance for the traits such as pod width, pod count per plant, pods within a cluster and seed count per pod. Cluster I consisted of genotypes with early maturity, as evidenced by their weaker average cluster values for both flowering and maturation period. On the flip side, cluster V and VI comprised genotypes the mature late.

The cluster means for the various attributes of broad bean are listed in Table 7. Cluster I exhibited the peak average values for attributes like plant height (103.22 cm), branch density (3.17), pod width (1.67 cm), cluster pod count (3.12), pods per individual plant (27.50), seed load per pod (2.83), 100 seed mass (129.83 g), seed yield (78.67 g), green pod yield (251.39 g), dry matter content (88.25%), ascorbic acid (18.24 mg/100g), protein (24.47%), carbohydrate (52.18%) and total soluble solids (8.15°Brix). Cluster IV had the top mean value for phenol (0.18%). Cluster V showed the greatest mean score for time to 50% bloom (62.51 days), maturity time (162.50 days) and moisture (14.50%). Cluster VI exhibited the longest pod length (12.09 cm).

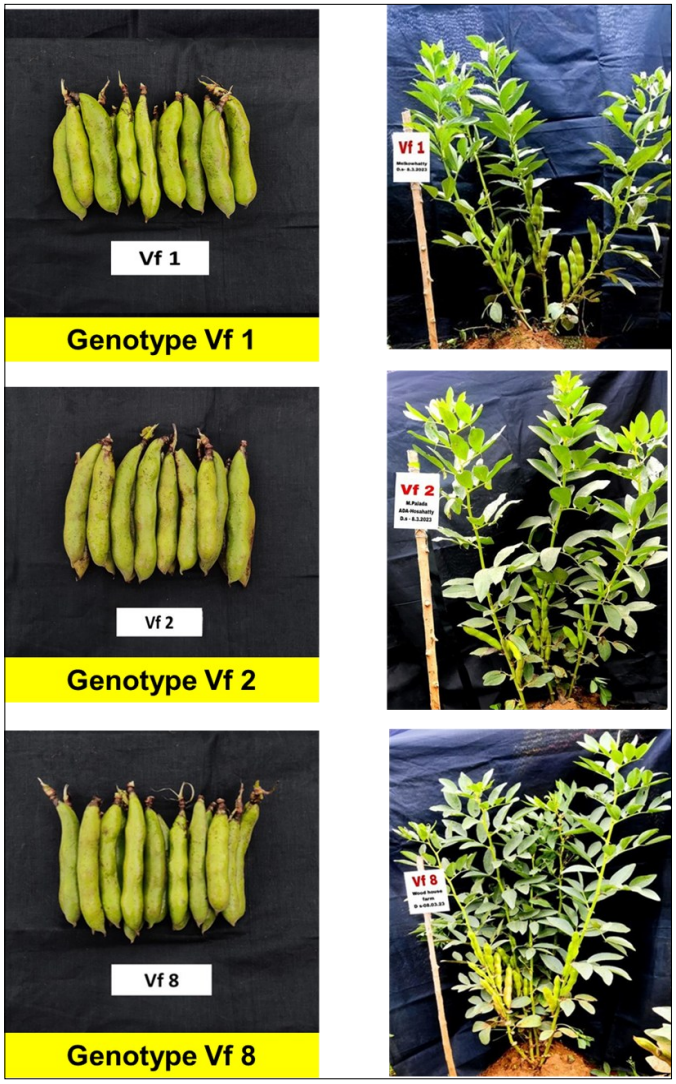

Fig. 3. Promising broad bean genotypes

The cophenetic correlation coefficient serves as a means of assessing the effectiveness of different clustering methods. A value approaching 1 signifies a highly accurate solution. The cophenetic index in this study was assessed as 0.672, indicating the effectiveness of the clustering pattern. The current work aligns with the earlier report on broad bean (29). From the results of the cluster analysis, the genotypes from cluster I namely, Vf 1, Vf 2 and Vf 8 are highly suitable for future hybridization programs as parental lines, in light of their superior performance in critical traits such as green pod yield, pod count, seed yield, protein and carbohydrate content.

Table 7. Cluster mean values for various traits in broad beans

Cluster	PH	BD	DF	MP	PL	PW	PCC	PCP	SCP	SWT	SY	PY	MT	DYM	ASC	PRO	PHE	CAR	TSS
I	103.22	3.17	55.61	152.33	11.38	1.67	3.12	27.50	2.83	129.83	78.67	251.39	11.75	88.25	18.24	24.47	0.14	52.18	8.15
II	97.55	2.36	58.13	155.79	10.79	1.56	2.36	16.36	2.71	107.64	64.93	152.43	13.14	86.86	16.64	20.71	0.16	47.88	7.87
III	100.62	3.15	57.66	155.12	10.97	1.64	2.38	17.50	2.75	119.25	74.44	199.88	12.69	87.31	15.52	21.64	0.13	50.52	7.85
IV	93.06	2.37	58.69	157.67	10.79	1.52	2.33	13.44	2.29	96.50	56.33	114.78	12.83	87.17	15.62	21.89	0.19	47.58	7.57
V	84.17	1.90	62.51	162.50	10.13	1.53	2.00	11.83	2.12	86.50	47.00	51.67	14.50	85.50	13.40	18.96	0.16	44.42	7.30
VI	73.50	2.20	60.90	160.00	12.09	1.52	2.08	12.92	2.00	99.75	45.12	91.67	13.75	86.25	17.41	20.48	0.17	45.50	7.10

PH- Plant height (cm), BD- Branch density, DF- Days to 50% flowering, MP- Maturity period, PL- Pod length (cm), PW- Pod width (cm), PCC- Pod count per cluster, PCP- Pod count per plant, SCP- Seed count per pod, SWT- 100 seed weight (g), SY- Seed yield (g), PY- Pod yield (g), MT- Moisture (%), DYM- Dry matter (%), ASC- Ascorbic acid (mg/100g), PRO- Protein (%), PHE- Phenol (%), CAR- Carbohydrate (%), TSS- Total soluble solids (°Brix)

Molecular Variability using SSR primers

The broad bean strains acquired were subjected to molecular analysis using SSR markers to assess the variability at the genetic level. Initially, a total of thirty different SSR primers were assessed to determine their capacity to produce distinct polymorphisms. Out of all the markers that were tested, only 16 markers (Table 2), yielded yield polymorphic alleles and were subsequently analysed further. The amplified band was counted based on their clarity and molecular weight relative to the DNA Ladder (Fig. 4). The analysis included determining the amplicon range, allele count, observed heterozygosity, expected heterozygosity, PIC value and Shannon index (Table 8). Across the 20 genotypes, a total of 43 consistent alleles were identified, with molecular weights ranging from 90 to 700 base pairs. The allele number vary between 2 and 6, averaging 2.68 per locus. Markers that exhibit a high number of alleles are regarded as highly informative and potent markers.

The efficacy of a marker can be quantified through various statistical methods, especially observed heterozygosity, expected heterozygosity, Polymorphism Information Content and Shannon index, all of which were employed in this study to evaluate the effectiveness of the SSR primers (Table 8). The PIC value measures the informativeness of a genetic marker. A marker is considered highly informative when its PIC value exceeds 0.5 (30). The PIC values ranged from 0.188 (GBSSR-VF-119) to 0.660 (GBSSR-VF-172), with an average PIC value of 0.402. Observed heterozygosity shows actual genetic variation, whereas expected heterozygosity predicts it under Hardy-Weinberg equilibrium. Observed heterozygosity (Ho) ranged from 0.116 (VfG 41) to 0.473 (CAAS7) averaging 0.340, whereas expected heterozygosity (He) stretched from 0.188 (GBSSR-VF-119 and VfG 41) to 0.493 (GBSSR-VF-175), showing moderate genetic diversity with limited genetic variation. The Shannon information index is a metric of diversity that evaluates both the abundance and uniformity of species or genes within a dataset. Low Shannon index values indicate reduced diversity, often due to dominance by one or a few genes, while higher values signify greater diversity with broader range of genes. The highest Shannon information index (I) was found to be 0.598 (GBSSR-VF-131, VfG 44 and VfG 9). The lowest value was noticed for VfG 41 (0.197) with an average of 0.489. The findings echo previous research (31, 32). GBSSR-VF-172 demonstrated high informativeness, owing to its efficiency in detecting genetic disparity within different broad bean genotypes. These diversity indices reflect the capacity of the SSR primer to distinguish between various accessions.

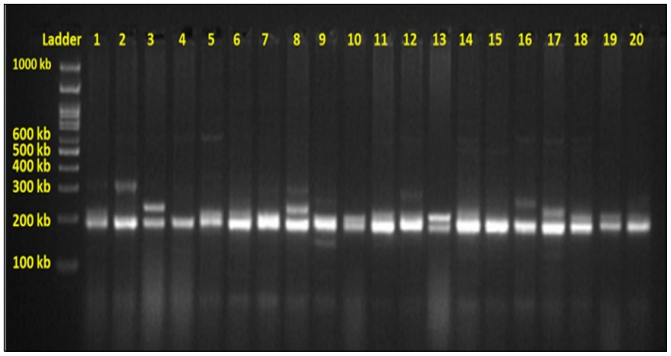

Fig. 4. Sample SSR profiling pattern of 20 genotypes of broad bean with GBSSR-VF-175 primer

Table 8. Details of banding pattern and discriminative statistics obtained with SSR markers

S. No.	Primer name	Size (bp)	Na	Ho	He	PIC	I
1.	GBSSR-VF-172	190-210	3	0.291	0.407	0.660	0.459
2.	GBSSR-VF-175	190-240	2	0.388	0.493	0.548	0.576
3.	GBSSR-VF-131	220-230	2	0.409	0.486	0.500	0.598
4.	GBSSR-VF-168	240-260	3	0.286	0.380	0.545	0.442
5.	GBSSR-VF-119	370-410	2	0.250	0.188	0.188	0.347
6.	GBSSR-VF-28	190-700	6	0.265	0.308	0.307	0.412
7.	GBSSR-VF-20	180-680	4	0.280	0.354	0.358	0.418
8.	GBSSR-VF-115	150-170	2	0.394	0.444	0.420	0.579
9.	GBSSR-VF-113	290-300	2	0.366	0.375	0.420	0.544
10.	VfG 19	90-600	4	0.435	0.382	0.369	0.513
11.	VfG 1	100-130	3	0.284	0.264	0.333	0.441
12.	VfG 44	270-290	2	0.409	0.486	0.480	0.598
13.	VfG 9	120-130	2	0.409	0.486	0.418	0.598
14.	VfG 41	190-230	2	0.116	0.188	0.228	0.197
15.	CAAS5	110-130	2	0.394	0.444	0.375	0.579
16.	CAAS7	190-200	2	0.473	0.465	0.280	0.408
	Average		2.688	0.340	0.384	0.402	0.489

Size (bp)- Amplified product size, Na- Allele number, Ho- Observed heterozygosity, He- Expected heterozygosity, PIC- Polymorphism Information Content, I- Shannon diversity index

Cluster Analysis based on SSR primers

The resulting Jaccard's Similarity matrix from binary data quantifies the genetic similarity among different broad bean genotypes as defined by SSR markers. Higher values indicate a stronger genetic resemblance, while lower values signify a weaker resemblance. In other words, genotypes with values approaching 1 demonstrate a higher level of genetic similarity, whereas those approaching 0 indicate a lower level of similarity. The Jaccard Coefficient spanned from 0.207 to 0.727. Striking similarity (0.727) was evident between Vf 6 and Vf 16, which meant to be closely related genotypes. The genotypes Vf 17 and Vf 1 exhibited comparatively lower levels of similarity (0.207) which are considered to be more divergent. Comparable findings have been reported in previous studies (33, 34).

The dendrogram obtained using SSR primers revealed that the twenty genotypes were assigned to two prominent clusters based on their proximity (Fig. 5), which failed to match the earlier dendrogram based on morphological and biochemical data (Fig. 2). The main cluster I has the largest set of genotypes (15 genotypes). The cluster I was subdivided into two major clusters 1A and 1B. The cluster 1A is further subdivided into three sub-clusters A1 (includes genotypes Vf 16, Vf 6, Vf 7 and Vf 2), A2 (includes genotypes Vf 14, Vf 8 and Vf 11) and A3 (includes genotypes Vf 19 and Vf 10). The cluster 1B was classified into two sub-clusters B1 (includes genotypes Vf 4, Vf 1, Vf 20 and Vf 13) and B2 (includes genotypes Vf18 and Vf 15). The cluster II had five genotypes namely Vf 12, Vf 9, Vf 5, Vf 17 and Vf 3. The high yielding genotypes namely Vf 8, Vf 2, Vf 19, Vf 9 and Vf 1 were placed in different clusters. This suggests a significant level of genetic diversity between different genotypes under evaluation. Consequently, it will be used in the future breeding programme for broad beans, particularly for hybridization, where genotypes sourced from multiple groups will provide the highest level of heterosis, which is beneficial for yield. The dendrogram constructed illustrates the genetic linkages between the genotypes based on SSR markers.

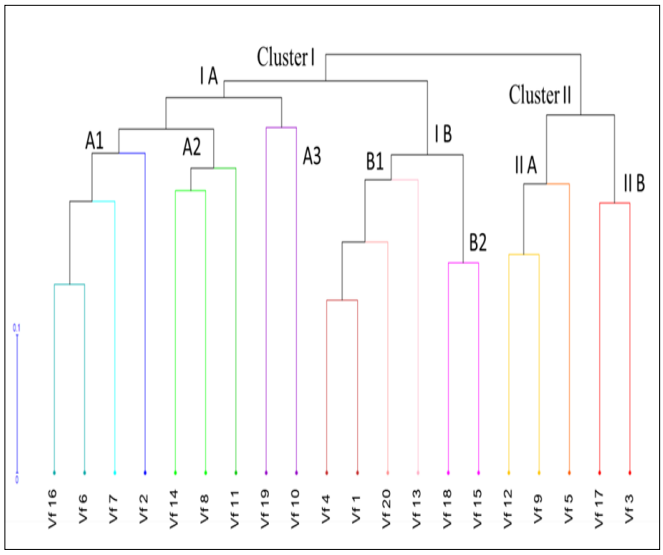

Fig. 5. Dendrogram based on SSR fingerprinting

Conclusion

Our investigation uncovered a significant level of genetic variability across all studied traits. The results of correlation and path analysis suggest that seed yield, 100-seed weight and pod count per plant are the main yield contributing traits, showing a strong positive direct effect and significant correlation with pod yield. Given their high heritability and genetic advancement, direct selection of these traits could effectively enhance pod yield in broad bean. Leveraging diverse genotypes from distinct clusters in hybridization programs could lead to the development of elite cultivars. Markers GBSSR-VF-172, GBSSR-VF-175 and GBSSR-VF-168 were identified as optimal for diversity analysis in broad bean due to their high heterozygosity and polymorphism information content. Both morpho-biochemical and molecular characterizations confirmed the genetic diversity within broad bean genotypes. This variability opens up opportunities to select superior genotypes, thereby enhancing the crop's overall productivity and trait diversity.

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Authors' contributions

AAR led the research experiments and authored the manuscript. SPT crafted the study design, facilitated genotype collection, and expertly supervised the entire experiment. SG contributed to the statistical analysis, interpretation and molecular characterization. SK and PR were involved in conducting and supervising the experiments. All authors reviewed, revised and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: The authors have no competing interests to disclose.

Ethical issues: None.

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