



RESEARCH ARTICLE

Morphological and molecular characterisation of broad bean (*Vicia faba* L.) genotypes for assessing diversity in yield and associated traits

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Abstract

Broad beans are one of the most important but neglected vegetable crops with excellent nutritional properties. The potential of broad beans can be utilised by developing improved cultivars with higher yields. There is a need to explore the available genetic diversity of broad beans and utilise them in improvement programs to promote their widespread adoption. The current study included 22 genotypes of broad bean along with 2 checks. Morphological diversity was assessed through the evaluation of 16 quantitative variables and 8 qualitative descriptors. The analysis revealed a wide range of genetic variability among the 22 genotypes. For molecular characterisation, 18 simple sequence repeat (SSR) markers were used to study the diversity among the tested genotypes. Two genotypes (RPCAU/BB/2023/11 and RPCAU/BB/2023/13) were found to be significantly superior to the checks for fresh pod yield per plant and its associated traits. The highest polymorphic information content (PIC) was recorded in M16 and M25 (0.84), while the lowest PIC was seen in M17 (0.24). A total of 82 alleles were obtained, of which 72 were shared alleles and 10 were unique alleles. The dendrogram formed 2 clusters, each containing 12 genotypes, which were further divided into 2 sub-clusters. The 22 genotypes showed high variability and can be further utilised in breeding programmes.

Keywords: genetic diversity; molecular and simple sequence repeat; morphological markers; qualitative; quantitative; yield traits

Introduction

The broad bean (*Vicia faba* L.), belonging to the family Fabaceae, is an annual leguminous crop with the chromosome number of $2n = 2x = 12$. Broad bean is a crop with multiple utilities that can be consumed as a vegetable or pulse and can also be grown as fodder, cover crop and green manure (1). A wide variation in the protein content (20–41 %) has been reported (2). It originated from the Mediterranean region, Egypt, North America and North-West Asia, from where it spreads to Europe, China, Japan and India (3). In India, broad bean cultivation spans approximately 2.63 million hectares, yielding roughly 4.87 million tonnes, with an average productivity of 2.12 t/ha (4). Although precise figures regarding the cultivated area in Bihar are lacking, the state holds the second position nationally in total bean production, with an output of 244550 tonnes. Furthermore, research trials have indicated yields of up to 3.61 t/ha for broad bean (5). Broad beans are an annual herb that can grow to a height of 0.3–2 m and possesses hollow, upright stems. It has alternate pinnate leaves with 2–6 leaflets

exhibiting narrow, broad and intermediate shapes. The flowers are borne in axillary racemes and are large and white with various melanin spots (greenish-yellow, brown and black) in the wings. They bear clusters of 3–5 flowers on short pedicels and flowers abundantly, but only a few flowers produce pods (5). The pods are short or long, depending on the variety. The seeds are brown, green, purple or black and may be flattened, angled or almost spherical. It is predominantly a self-pollinating crop, although cross-pollination ranging from 15 % to 20 % occurs through the activity of honeybees (6).

Despite being a highly nutritious crop, broad bean remains underutilised due to its limited scale cultivation. The lack of suitable technologies, improved varieties and cultivation practices are major constraints to the adoption of this crop. The available collections of faba bean are local landraces conserved by farmers and partly crossing the nature of the crops with their poor multiplication imparts insufficient *ex situ* conservation. For any crop improvement program to be successful, the presence of

variability is essential. Identifying superior genotypes adapted to specific locations in India would provide opportunities to enhance yield and stability (7). Duplication of plant materials is a major challenge in advanced crop improvement. Genetic analysis using molecular markers is one way to conserve germplasm. Simple sequence repeat (SSR)/microsatellite markers are highly polymorphic, co-dominant, have high allelic diversity and are easy to transfer (7). The present study was initiated to address the need for improved *V. faba* varieties by integrating morphological observations with molecular-level DNA analysis. This approach helps identify high-yielding, resilient genotypes and provides breeders with the essential data required to develop superior varieties capable of maintaining productivity under environmental stress, thereby supporting broader food security goals.

Materials and Methods

The field research was conducted at the Vegetable Research Farm of Dr. Rajendra Prasad Central Agricultural University, Pusa, Bihar, during the rabi season of 2023–24. The experimental materials comprised 22 different genotypes of broad bean collected from Manipur and Bihar, along with 2 checks, namely Pusa Sumeet and Pusa Udit. The experiments were conducted by following the randomised block design (RBD) with 3 replications.

Morphological characterisation

For the collection of morphological data, 5 randomly selected plants from each genotype were used. Data on 16 quantitative characters such as days to germination, plant height (cm), number of stems, number of pods per plant, number of leaflets per leaf,

first flowering node, days to first flowering, days to 50 % flowering, days to first pod formation, pod length (cm), pod width (cm), pod weight (g), number of seeds per pod, days to full pod development, seed weight (g) and yield of fresh pod per plant (g) were recorded at 15 days interval. Data on 8 qualitative characters, including growth habit, anthocyanin colouration of stem, leaf shape, melanin spot on wing, seed shape, colour of testa in dry seed and black pigmentation of hilum in dry seed, were also recorded and analysed.

Molecular characterisation

DNA extraction

DNA was extracted from the young leaves of broad bean plants using the cetyl trimethyl ammonium bromide (CTAB) extraction method as suggested by Doyle (8) with a few modifications. The CTAB-based DNA extraction method for fresh plant tissue uses cetyltrimethylammonium bromide to selectively precipitate DNA while removing polysaccharide and phenolic contaminants, thereby producing a high-quality template suitable for molecular studies (8).

Qualitative analysis of DNA

The qualities of isolated plant DNA was assessed on 1 % agarose gel following the method suggested in the previous study (9).

Simple sequence repeat marker used

Eighteen SSR markers (Table 1), which were procured from G-Bioscience, were used for the molecular analysis (10, 11). Due to their polymorphic nature and co-dominant properties, SSR markers were employed to accurately map the genetic distance among the genotypes (7).

Table 1. List of simple sequence repeat (SSR) markers used for molecular characterisation of broad beans genotypes

Primers		Forward (5' to 3')/Reverse (5' to 3')	Putative function
M16	F	AACCTCTCCGTTAACCAACC	Hypothetical protein, partial
	R	AACCTCGCGCTAGATAACA	
M10	F	CCATGGTTACTGCACTCGAA	Tyrosine protein kinase, active site
	R	GCCGTGCGATTGATTCGTATT	
M17	F	CTCCAACGAAGGCAGAGAAG	Histone H2B.3
	R	CATGATTCCCATAGCCTTGC	
M22	F	TCGCAATAGCACAGAACCTG	Predicted protein
	R	GATCAAACTCCCCAACCTCA	
M25	F	CGTCGTGAAAATCATGGAGA	Dehydrin DHN2
	R	CATTATTATTACACCGCCTCA	
M9	F	AATCACAGCGACGACGAC	Defender against cell death 1 (DAD-1)
	R	GCGGAATATGCAGACCAAT	
M37	F	TCTCAGCAACATTCCAAG	BAH domain-containing protein
	R	TTTTCCGAAACCTCA	
M43	F	TTCTGATGAGAAGCCAACCA	ADR12
	R	GGGGGAGGAACCAAGATTATT	
M46	F	GGATGGATTGATTCTCAACA	Defensin-like protein
	R	GCATAACTAACACATTATGCAGGA	
M58	F	ACATCAGGGAAGAACGCATC	Cap-binding protein-like protein
	R	GAGGGTGAAGACCAGCTTTG	
M57	F	TGCAGAGAAGCTAAGCACCA	Seed maturation protein
	R	TCGCATGGTACAGTAGCAAAA	
P11	F	CGTGGTTGATTGCTTC	Dehydration-induced protein ERD15
	R	ACCTCCATCTTCGCCTC	
M30	F	GTGTAAGTGAAGGAGTGAAT	Gamma-glutamyl cysteine synthetase precursor
	R	CGGAAAACAGTAGCCAT	
M41	F	CAACGCGGCAGTTAAAGAAT	Seed maturation protein
	R	CAGGTATGGCTGACACCTCA	
P121	F	ACCAGGCAGAGTTAGATAAGCA	Seed maturation protein
	R	GTCACCTCGGATGTCCAGGTAT	
P41	F	ACACTGTTCAACGGTAT	Fabatin precursor
	R	GGAGTGATTAGAAGGTAG	
P27	F	CGGGTTTATTCCATCATTT	BAH domain-containing protein
	R	CGTTATTGTTGTCGCTATTT	
P139	F	AACCCATCTGGAAGAAACA	SPX domain gene 4 (SPX4)
	R	TTGAGAATCCGAAGAAACC	

Amplification and agarose gel electrophoresis of genomic DNA

Primer dilution for simple sequence repeat-polymerase chain reaction (SSR-PCR)

To prepare the stock primer solution, 10 µL of primer was mixed with 90 µL of nuclease-free water. The diluted primers obtained from this process were then preserved at -20 °C for subsequent use. Simple sequence repeat amplification was conducted in a thermal cycler (Agilent Technologies), which included 1 µL of template DNA, 5 µL of Mastermix, 0.5 µL of reverse primer, 0.5 µL of forward primer and 3 µL nuclease-free water for each reaction with a total volume of 10 µL. For a total of 22 reactions, the components were assembled in individual 1.5 mL Eppendorf tubes by combining 78 µL of molecular-grade water and 130 µL of Mastermix and the resulting mixture was maintained on ice. Finally, 1 µL of the diluted DNA template was dispensed into each PCR tube. Upon concluding these procedures, 13 µL of both forward and reverse primers were added and gently vortexed to facilitate proper mixing. Subsequently, 9 µL was dispensed into each PCR tube, followed by a light vortex and the PCR reaction was initiated by the following specific temperatures for denaturation, annealing and extension.

Agarose gel electrophoresis

For gel electrophoresis, 3 % agarose gel was prepared by melting the agarose in an oven and then allowing it to cool to a bearable temperature, after which 5 µL of ethidium bromide was added. The gel was then poured on the gel tray and allowed to solidify. The PCR product was loaded into the designated wells and run along with a 50 bp ladder. The gel was subjected to an electric current of 80 V for a duration of 50 min. The dye migrated to three-quarters of the gel's length. Visualisation of the gel was performed using a gel documentation system and the resulting images were preserved for subsequent analysis.

Scoring and data analysis

The genotype banding pattern produced by each set of primers was scored separately. The presence of a band for a specific amplicon size was assigned a score of one, whereas the absence of any band at that location was scored as zero. The allele size of several markers was calculated using the banding pattern of the 50 bp ladder. Data analysis was conducted using Darwin 6.0 software (12).

Polymorphic information content

Polymorphic information content (PIC) was used to refer to the informativeness of microsatellites by using the formula:

$$PIC = 1 - (\sum p_i^2)$$

Where, P_i = the i^{th} allele repetition, a metric commonly associated with heterozygosity and genetic variation (13). The polymorphism percentage was calculated using data from SSR analysis and dividing the amplified polymorphic band by the total number of bands seen.

Unweighted pair group method with arithmetic mean

The unweighted pair group method with arithmetic mean (UPGMA) represents a straightforward method for constructing phylogenetic trees. Its primary aim is to develop taxonomic phenograms that illustrate the relationships among operational taxonomic units (OTUs). The process of successive clustering

assesses local compatibility among OTUs based on their similarity, thereby facilitating a gradual expansion of the tree. Initially, the 2 most closely related OTUs are identified and combined into a new "composite" OTU. The next step involves selecting the pair of OTUs that demonstrates the greatest similarity within the newly established group for additional clustering. This process continues until only closely related OTUs are retained. During each iteration, the 2 most proximate clusters are combined into a more comprehensive grouping. The distance between any 2 clusters, designated as A and B, is determined by calculating the average of all distances between elements "x" in cluster A and elements "y" in cluster B, or by computing the mean distance among the elements within each respective cluster.

$$\frac{1}{|A| \cdot |B|} \sum_{y \in B} \sum_{x \in A} d(x, y)$$

Results

Morphological characterisation

Qualitative traits

The average data of all 8 qualitative parameters of the 22 genotypes studied are shown in Table 2. The growth habit of the genotypes was recorded as determinate, semi-determinate and indeterminate. Genotypes recorded as indeterminate include RPCAU/BB/2023/01, RPCAU/BB/2023/02, RPCAU/BB/2023/03, RPCAU/BB/2023/20, RPCAU/BB/2023/1 and RPCAU/BB/2023/22. Pusa Sumeet and Pusa Udit were recorded as semi-determinate. No genotypes exhibited determinate growth habits. Anthocyanin colouration in the stem was recorded as present or absent. Anthocyanin colouration in the stem was found to be present in RPCAU/BB/2023/01, RPCAU/BB/2023/02 and RPCAU/BB/2023/03. However, the genotype RPCAU/BB/2023/07, RPCAU/BB/2023/08 and RPCAU/BB/2023/10, exhibits no anthocyanin colouration in the stem. The leaf shape was recorded as narrow, intermediate and rounded. Intermediate leaf shape was recorded in RPCAU/BB/2023/01, RPCAU/BB/2023/02 and RPCAU/BB/2023/03. Narrow-leaf shape was recorded in RPCAU/BB/2023/05, RPCAU/BB/2023/08 and RPCAU/BB/2023/15. A melanin spot in the wing was present in all the genotypes. Seed shapes were recorded as flattened, round and angular in the genotypes. Flattened seed shape was recorded in the genotypes RPCAU/BB/2023/01, RPCAU/BB/2023/02 and RPCAU/BB/2023/03. Angular seed shape was recorded in the RPCAU/BB/2023/06, RPCAU/BB/2023/12 and RPCAU/BB/2023/14. Round seed shape was found to be present in RPCAU/BB/2023/05, RPCAU/BB/2023/07 and RPCAU/BB/2023/08. Light brown testa colour in dry seed was recorded in RPCAU/BB/2023/01, RPCAU/BB/2023/03 and RPCAU/BB/2023/06. Light green colour in dry seed was recorded in RPCAU/BB/2023/02, RPCAU/BB/2023/04 and RPCAU/BB/2023/11. Black colour testa colour in dry seed was recorded in RPCAU/BB/2023/05 and RPCAU/BB/2023/08. Deep brown testa colour in dry seed was recorded in RPCAU/BB/2023/09, RPCAU/BB/2023/10 and RPCAU/BB/2023/12. Black pigmentation of the hilum in dry seed was seen and recorded as present or absent. Black pigmentation of the hilum in dry seed was present in RPCAU/BB/2023/05, RPCAU/BB/2023/07 and RPCAU/BB/2023/08. Black pigmentation of the hilum in dry seed was recorded to be absent in RPCAU/BB/2023/01, RPCAU/BB/2023/02 and RPCAU/BB/2023/03.

Table 2. Morphological characterisation for qualitative traits of different genotypes of broad bean

Genotypes	Growth habit	Anthocyanin colouration of the stem	Leaf shape	Seed shape	Melanin spot in the wing	Anthocyanin colouration of the standard in the flower	Colour of testa in Black pigmentation of the hilum in dry seed	Absent
RPCAU/BB/2023/01	Indeterminate	Present	Intermediate	Flat	Present	Present	Light brown	Absent
RPCAU/BB/2023/02	Indeterminate	Present	Intermediate	Flat	Present	Present	Light green	Absent
RPCAU/BB/2023/03	Indeterminate	Present	Intermediate	Flat	Present	Present	Light brown	Absent
RPCAU/BB/2023/04	Indeterminate	Present	Intermediate	Flat	Present	Present	Light green	Absent
RPCAU/BB/2023/05	Indeterminate	Present	Narrow	Round	Present	Present	Black	Present
RPCAU/BB/2023/06	Indeterminate	Present	Intermediate	Angular	Present	Present	Light brown	Absent
RPCAU/BB/2023/07	Indeterminate	Absent	Intermediate	Round	Present	Present	Light brown	Present
RPCAU/BB/2023/08	Indeterminate	Absent	Narrow	Round	Present	Present	Black	Present
RPCAU/BB/2023/09	Indeterminate	Present	Intermediate	Flat	Present	Present	Deep brown	Absent
RPCAU/BB/2023/10	Indeterminate	Absent	Intermediate	Round	Present	Present	Deep brown	Present
RPCAU/BB/2023/11	Indeterminate	Absent	Intermediate	Round	Present	Present	Light green	Present
RPCAU/BB/2023/12	Indeterminate	Present	Intermediate	Angular	Present	Present	Deep brown	Absent
RPCAU/BB/2023/13	Indeterminate	Present	Intermediate	Flat	Present	Present	Light brown	Present
RPCAU/BB/2023/14	Indeterminate	Present	Intermediate	Angular	Present	Present	Deep brown	Present
RPCAU/BB/2023/15	Indeterminate	Present	Narrow	Angular	Present	Present	Light brown	Absent
RPCAU/BB/2023/16	Indeterminate	Present	Intermediate	Flat	Present	Present	Light green	Absent
RPCAU/BB/2023/17	Indeterminate	Present	Intermediate	Round	Present	Present	Deep brown	Present
RPCAU/BB/2023/18	Indeterminate	Absent	Narrow	Round	Present	Present	Light brown	Present
RPCAU/BB/2023/19	Indeterminate	Absent	Intermediate	Round	Present	Present	Deep brown	Present
RPCAU/BB/2023/20	Indeterminate	Present	Narrow	Angular	Present	Present	Deep brown	Absent
RPCAU/BB/2023/21	Indeterminate	Absent	Intermediate	Round	Present	Present	Light green	Present
RPCAU/BB/2023/22	Indeterminate	Absent	Narrow	Round	Present	Present	Light green	Present
Pusa Sumeet (Check)	Semi-determinate	Absent	Intermediate	Round	Present	Present	Light brown	Present
Pusa Udit (Check)	Semi-determinate	Absent	Intermediate	Flattened	Present	Present	Light brown	Present

Quantitative traits

The average data of all 16 quantitative parameters of the 22 genotypes studied are shown in Table 3. The maximum number of days (9.00) to seed germination were recorded in the genotypes RPCAU/BB/2023/11 and RPCAU/BB/2023/18. The minimum number of days (5.00) to seed germination after sowing was observed in line RPCAU/BB/2023/07, with an overall mean of 7.99 days. The overall average plant height recorded was 90.66 cm, with the tallest plant recorded in RPCAU/BB/2023/11 (125.37 cm), followed by RPCAU/BB/2023/10 (121.23 cm), showing significantly better results than the check varieties. The shortest plant height was recorded in RPCAU/BB/2023/15 (72.47 cm). The recorded range of the number of stems per plant was 4.73–7.00, with an overall mean of 6.22. The minimum was recorded in RPCAU/BB/2023/03 (4.73)

and the maximum was recorded in RPCAU/BB/2023/07 (7.00), which is on par with the check variety, Pusa Sumeet (7.00), but higher than the check Pusa Udit (6.97). The average number of leaflets per leaf ranges from 5.08 to 6.93 in the genotypes RPCAU/BB/2023/21 and RPCAU/BB/2023/13, respectively. The overall mean for this character was 6.13. Among all the genotypes studied, the highest number of leaflets per leaf was recorded in RPCAU/BB/2023/13 (6.99), which exhibited significant superiority over both the check variety, Pusa Sumeet (5.70) and Pusa Udit (5.60).

The minimum node to first flowering was recorded in the genotype RPCAU/BB/2023/17 (4.80), followed by RPCAU/BB/2023/16 (4.83), which was lower and significantly better than the check varieties, Pusa Sumeet and Pusa Udit. The maximum node to first flowering was recorded in RPCAU/BB/2023/15 (9.60), with a

Table 3. Mean performance of tested genotypes of broad bean

Genotypes	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
RPCAU/BB/2023/01	8.33	83.10	5.60	7.90	6.50	50.87	61.00	80.33	11.20	2.12	11.13	14.90	3.00	96.00	84.03	165.23
RPCAU/BB/2023/02	8.67	89.30	6.00	8.20	6.07	50.30	60.67	80.63	11.43	2.22	10.86	19.93	3.00	102.40	97.10	215.37
RPCAU/BB/2023/03	7.67	83.40	4.73	8.80	6.17	48.17	61.00	79.37	11.23	1.99	11.00	18.60	2.48	94.90	95.00	204.22
RPCAU/BB/2023/04	7.33	86.07	5.90	7.50	5.27	45.47	61.33	75.67	9.33	1.93	9.91	16.00	2.93	104.67	72.13	157.41
RPCAU/BB/2023/05	7.00	80.73	6.00	7.37	6.50	45.17	63.50	82.27	8.23	1.34	7.17	24.07	2.73	100.57	65.37	172.54
RPCAU/BB/2023/06	8.00	82.30	6.20	7.47	6.83	43.50	76.00	88.33	8.47	1.53	5.99	19.80	2.87	101.50	73.00	117.57
RPCAU/BB/2023/07	5.00	94.97	7.00	6.60	5.93	46.50	77.50	91.50	7.97	1.33	5.22	24.47	3.50	105.40	52.33	127.26
RPCAU/BB/2023/08	8.67	100.10	5.63	5.83	5.90	45.13	74.67	82.50	8.33	1.33	6.71	29.30	4.00	106.87	63.20	195.58
RPCAU/BB/2023/09	8.00	88.60	6.10	6.10	6.20	45.67	62.00	82.00	9.50	1.99	11.89	15.80	3.37	97.07	78.43	186.17
RPCAU/BB/2023/10	7.67	121.23	6.93	6.63	6.33	44.58	76.50	84.17	7.37	1.43	8.37	28.20	3.67	105.67	46.73	235.15
RPCAU/BB/2023/11	9.00	125.37	6.70	6.20	5.93	43.48	77.00	84.00	9.63	1.33	9.01	31.00	3.63	107.47	61.63	278.83
RPCAU/BB/2023/12	8.00	110.73	5.27	7.33	6.47	44.83	60.00	80.33	11.63	2.03	10.80	16.20	3.73	92.37	84.33	173.39
RPCAU/BB/2023/13	8.00	100.27	4.87	5.40	6.93	33.40	55.60	65.47	11.17	2.17	12.82	19.90	2.67	72.83	89.47	254.72
RPCAU/BB/2023/14	7.67	77.33	6.00	6.60	6.83	46.17	64.50	81.33	11.43	1.57	10.12	17.40	3.40	105.83	61.80	173.46
RPCAU/BB/2023/15	8.00	72.47	6.20	9.60	6.07	50.33	82.00	86.33	8.23	1.33	7.32	24.27	3.33	104.47	68.07	166.89
RPCAU/BB/2023/16	7.00	82.73	6.27	4.83	6.50	50.17	63.33	80.33	11.23	2.15	11.41	15.80	2.93	101.50	64.60	178.61
RPCAU/BB/2023/17	8.00	76.67	6.80	4.80	6.33	55.67	69.67	83.00	6.47	1.17	2.78	31.93	3.65	106.07	27.77	88.36
RPCAU/BB/2023/18	9.00	81.33	6.87	6.00	5.80	43.13	62.33	83.33	6.10	1.21	3.17	23.93	2.83	104.27	22.50	75.83
RPCAU/BB/2023/19	8.67	75.20	6.53	5.00	6.13	45.17	73.33	78.17	6.30	1.20	3.04	53.13	3.00	106.03	27.43	160.76
RPCAU/BB/2023/20	8.33	90.67	6.93	8.87	6.67	54.50	82.33	91.83	8.43	1.22	5.21	21.40	3.80	112.00	62.70	111.46
RPCAU/BB/2023/21	7.67	95.33	6.33	4.87	5.07	44.83	66.67	80.50	6.53	1.16	4.53	34.33	3.45	103.47	27.43	155.05
RPCAU/BB/2023/22	8.67	83.00	6.40	5.40	5.47	45.50	68.33	83.33	6.50	1.11	5.43	34.40	3.60	105.63	25.97	186.31
Pusa Sumeet (Check)	9.00	100.47	7.00	6.00	5.70	48.00	78.67	90.83	6.30	1.22	5.50	23.67	3.07	106.33	32.40	129.74
Pusa Udit (Check)	8.33	94.47	6.97	5.80	5.60	48.17	76.00	86.67	6.27	1.39	5.05	30.20	3.30	98.90	28.77	151.81
Mean	7.99	90.66	6.22	6.63	6.13	46.61	68.91	82.59	8.72	1.56	7.69	24.53	3.25	101.76	58.84	169.24
Min	5.00	72.47	4.73	4.80	5.07	33.40	55.60	65.47	6.10	1.11	2.78	14.90	2.48	72.83	22.50	75.83
Max	9.00	125.37	7.00	9.60	6.93	55.67	82.33	91.83	11.63	2.22	12.82	53.13	4.00	112.00	97.10	278.83
SEm	0.38	6.41	0.31	0.33	0.29	0.94	1.36	2.69	0.42	0.08	0.68	1.18	0.21	4.77	1.07	9.98
CV (%)	8.25	12.24	8.74	8.60	8.26	3.51	3.42	5.63	8.27	8.45	15.32	8.32	10.97	8.11	3.14	10.22
CD 5 %	1.09	18.30	0.90	0.94	0.84	2.70	3.89	7.67	1.19	0.22	1.94	3.37	0.59	13.61	3.04	28.41
CD 1 %	1.45	24.35	1.19	1.25	1.11	3.59	5.17	10.21	1.58	0.29	2.58	4.48	0.78	18.12	4.06	37.93

1. Days to germination; 2. Plant height (cm); 3. No. of stems per plant; 4. No. of nodes up to first flowering; 5. No. of leaflets per leaf; 6. Days to 1st flowering; 7. Days to 50 % flowering; 8. Days to 1st pod formation; 9. Pod length (cm); 10. Pod width (cm); 11. Pod weight (g); 12. No. of pods/plant; 13. No. of seeds per pod; 14. Days to full pod formation; 15. Seed weight (g); 16. Yield of fresh pods per plant. CV: coefficient of variation; CD: critical difference.

mean value of 6.63. The maximum number of days taken to first flower appearance was recorded in RPCAU/BB/2023/17 (55.67), while the overall average number of days taken for first flower appearance was 46.61. The minimum number of days taken for the first flower appearance in the plant was recorded in RPCAU/BB/2023/13 (33.40), followed by RPCAU/BB/2023/18 (43.13), which is more significant than the check variety Pusa Sumeet (48.00) and Pusa Udit (48.17) for earliness. The maximum number of days taken for the 50 % flowering was recorded in RPCAU/BB/2023/20 (82.33). The minimum number of days was recorded for RPCAU/BB/2023/13 (55.60), followed by RPCAU/BB/2023/12 (60.00), which is significantly better for earliness than the check variety Pusa Sumeet (78.67) and Pusa Udit (76.00).

The minimum number of days taken to first pod formation was recorded in RPCAU/BB/2023/13 (65.47), followed by RPCAU/BB/2023/04 (75.67), which was significantly earlier than the check varieties, Pusa Sumeet (90.83) and Pusa Udit (86.67). The maximum number of days taken was recorded in RPCAU/BB/2023/20 (91.83) with an overall mean of 82.59. The pod length (cm) ranged from 6.10 (RPCAU/BB/2023/18) to 11.63 (RPCAU/BB/2023/12) with an average of 8.72. Seven genotypes namely RPCAU/BB/2023/12 (11.63), RPCAU/BB/2023/02 (11.43), RPCAU/BB/2023/14 (11.43), RPCAU/BB/2023/01 (11.20), RPCAU/BB/2023/03 (11.23), RPCAU/BB/2023/16 (11.23) and RPCAU/BB/2023/13 (11.17) were found to be significantly superior over the check variety, Pusa Sumeet (6.30) and Pusa Udit (6.27). The width of the pod (cm) varied from 1.11 (RPCAU/BB/2023/22) to 2.22 (RPCAU/BB/2023/02) and the mean value was 1.56. Genotypes, viz., RPCAU/BB/2023/01 (2.12), RPCAU/BB/2023/02 (2.22), RPCAU/BB/2023/03 (1.99), RPCAU/BB/2023/04 (1.93), RPCAU/BB/2023/06 (1.53), were found significantly superior over the check Pusa Sumeet (1.22) and Pusa Udit (1.39). The average pod weight (g) ranged from 2.78 (RPCAU/BB/2023/17) to 12.82 (RPCAU/BB/2023/13) with a mean value of 7.87. The genotypes such as RPCAU/BB/2023/01 (11.13), RPCAU/BB/2023/02 (10.86), RPCAU/BB/2023/03 (10.99), RPCAU/BB/2023/04 (9.91) and RPCAU/BB/2023/05 (7.72) outperformed the checks Pusa Sumeet (5.50) and Pusa Udit (5.050). The days taken to full pod development range from 72.83 (RPCAU/BB/2023/13) to 112.00 (RPCAU/BB/2023/20), with an average of 101.76 days. The genotypes, namely, RPCAU/BB/2023/09 (97.07), RPCAU/BB/2023/01 (96.00), RPCAU/BB/2023/03 (94.90), RPCAU/BB/2023/12 (92.37) and RPCAU/BB/2023/13 (72.83), with minimum duration, were found to be significant for earliness compared to check Pusa Sumeet (106.33) and Pusa Udit (98.90). The number of pods per plant ranged from 14.90 (RPCAU/BB/2023/01) to 53.13 (RPCAU/BB/2023/19) and the overall mean was 24.53. The 5 genotypes, RPCAU/BB/2023/19 (53.13), RPCAU/BB/2023/21 (34.33), RPCAU/BB/2023/22 (34.40), RPCAU/BB/2023/17 (31.93) and RPCAU/BB/2023/11 (31.00), were significantly superior over the checks, Pusa Sumeet (23.67) and Pusa Udit (30.20).

The highest number of seeds per pod was recorded in RPCAU/BB/2023/08 (4.00) and the minimum number of seeds was recorded in RPCAU/BB/2023/03 (2.48), with an average performance of 3.25. The genotypes namely, RPCAU/BB/2023/07 (3.50), RPCAU/BB/2023/08 (4.00), RPCAU/BB/2023/09 (3.37), RPCAU/BB/2023/10 (3.67), RPCAU/BB/2023/11 (3.63), RPCAU/BB/2023/12 (3.73), RPCAU/BB/2023/14 (3.40), RPCAU/BB/2023/15 (3.33), RPCAU/BB/2023/17 (3.65), RPCAU/BB/2023/20 (3.80), RPCAU/BB/2023/08 (3.45), RPCAU/BB/2023/22 (3.60) were significantly higher than the

check variety, Pusa Sumeet (3.07) and Pusa Udit (3.30). The highest seed weight (g) was observed in RPCAU/BB/2023/02 (97.10), followed by RPCAU/BB/2023/03 (95.00). The lowest seed weight was recorded in RPCAU/BB/2023/18 (22.50), with an average performance of 58.84. Over the check variety, all the genotypes were significantly superior except in the 5 genotypes, namely, RPCAU/BB/2023/17 (27.77), RPCAU/BB/2023/18 (22.50), RPCAU/BB/2023/19 (27.43), RPCAU/BB/2023/21 (27.43) and RPCAU/BB/2023/22 (25.97).

The total yield of fresh pod per plant (g/plant) varied from 75.83 (RPCAU/BB/2023/18) to 278.8 (RPCAU/BB/2023/11) with a mean of 169.24. The genotypes RPCAU/BB/2023/01 (165.23), RPCAU/BB/2023/02 (215.37), RPCAU/BB/2023/03 (204.22), RPCAU/BB/2023/04 (157.41), RPCAU/BB/2023/08 (195.58), RPCAU/BB/2023/09 (186.17), RPCAU/BB/2023/10 (235.15), RPCAU/BB/2023/11 (278.83), RPCAU/BB/2023/12 (173.39), RPCAU/BB/2023/13 (254.72), RPCAU/BB/2023/14 (173.46), RPCAU/BB/2023/15 (166.89), RPCAU/BB/2023/16 (178.61), RPCAU/BB/2023/19 (160.76), RPCAU/BB/2023/21 (155.05) and RPCAU/BB/2023/22 (186.31) were found to be substantially better over the check Pusa Sumeet (129.74) and Pusa Udit (151.81).

Molecular characterisation

In the present investigation, SSR markers were used to study the genetic diversity among the 22 broad bean genotypes. The results of molecular characterisation of different genotypes under study were presented in the following headings.

Amplification of genomic DNA by using primers

Primer pairs amplify DNA segments of varied length, yielding alleles. Each primer pair amplifies DNA fragments of varied sizes; the resulting pieces can be recorded as alleles. The number of unique alleles represents the alleles specific to a single primer pair, whereas the number of shared alleles indicates how many alleles are shared among different primer pairs. M16 primers amplified DNA fragments ranging in size from 85 to 223 base pairs. It was noticed that there are 7 different alleles found amongst the tested genotypes. Out of them, no allele was unique to this primer and 7 were shared with other primers. The PIC value is high with 0.84. M10 primers amplified DNA fragments ranging in size from 203 to 268 base pairs. It detected 4 different alleles in total; no allele was unique to this primer and 4 were shared with other primers. The PIC value was recorded as high at 0.66. M17 primers amplified DNA fragments ranging in size from 183 to 200 base pairs. A total of 2 different alleles were detected. Among them, no allele was unique to this primer and both were shared with other primers. The PIC value was low (0.24). M30 primers amplified DNA fragments ranging in size from 179 to 200 base pairs. A total of 2 distinct alleles were detected, with no unique alleles and both alleles were shared with other primers. The PIC value was moderate (0.46).

Scoring of the alleles and the similarity assessment

A total of 82 alleles were obtained, of which 72 were shared alleles and 10 unique allelic variants generated as amplified product by PCR using 18 primers (Table 4). The highest number of shared alleles per locus was recorded in primer M16, whereas the number of unique alleles was recorded with primer M58. The highest similarity coefficient was detected between RPCAU/BB/2023/22 and Pusa Sumeet with a value of 0.083, whereas the highest

Table 4. Polymorphic information content of simple sequence repeat (SSR) primers

SSR primers	Amplicon size		Amplicon size difference	Number of unique alleles	Number of shared alleles	Total no. of alleles	No. of bands	PIC
	Max.	Min.						
M16	223	85	138	0	7	7	23	0.84
M10	268	203	65	0	4	4	24	0.66
M17	200	183	17	0	2	2	22	0.24
M22	175	132	43	0	4	4	22	0.63
M25	328	185	143	0	6	6	22	0.84
M9	223	102	121	0	5	5	23	0.74
M37	261	167	94	1	5	6	19	0.76
M43	209	112	97	1	3	4	21	0.62
M46	198	117	81	1	3	4	22	0.64
M58	234	112	122	2	5	7	22	0.81
M57	285	189	96	1	3	4	22	0.53
P11	180	162	18	0	3	3	23	0.57
M30	200	179	21	0	2	2	22	0.46
M41	223	85	138	1	6	7	28	0.82
P121	170	90	80	2	4	6	22	0.73
P41	146	95	51	0	5	5	22	0.78
P27	229	184	45	1	3	4	23	0.48
P139	121	107	14	0	2	2	20	0.42
Total				10	72	82	402	-

SSR: simple sequence repeat; PIC: polymorphic information content.

dissimilarity was observed between RPCAU/BB/2023/15 and RPCAU/BB/2023/04 with a 0.05 value.

Polymorphism information content

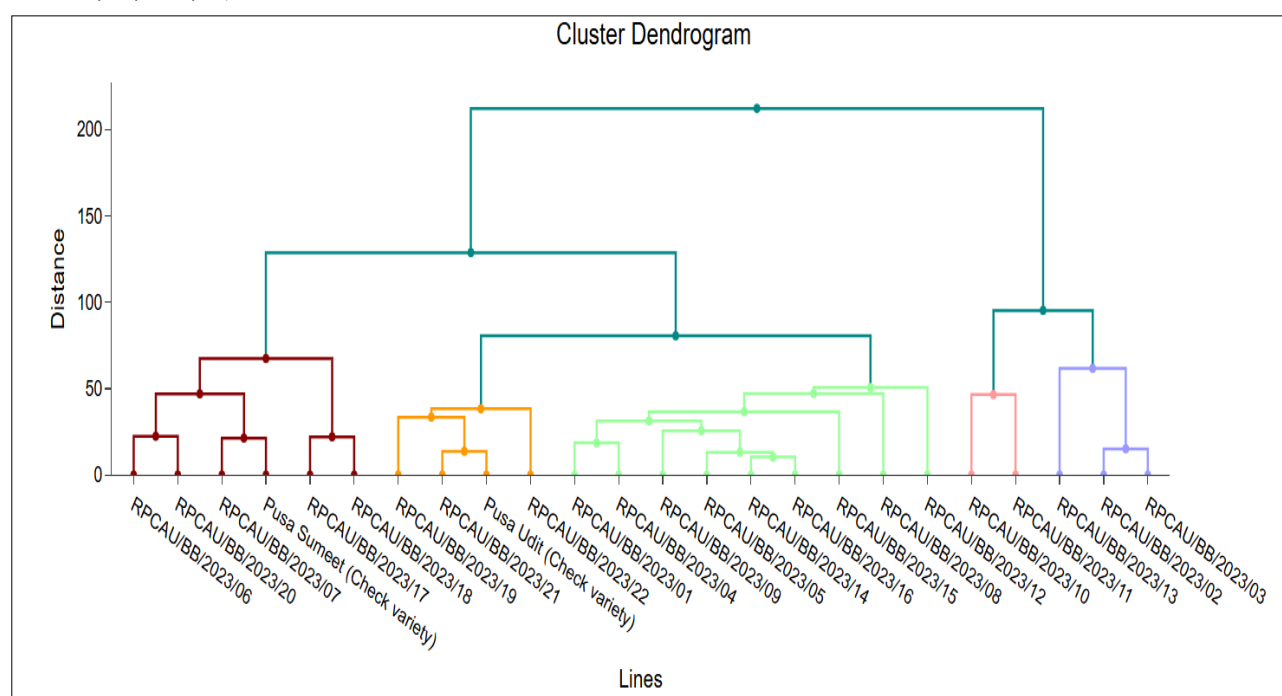
The PIC values range from 0.24 (M17) to 0.84 (M16 and M25). The highest PIC was recorded in primer M16 and M25 (0.84), followed by M14 (0.82) and M48 (0.81).

Cluster formation based on markers

A total of 2 clusters were formed (Fig. 1) and these clusters were sub-grouped into 2 clusters. Cluster IA consists of Cluster RPCAU/BB/2023/04, RPCAU/BB/2023/05, RPCAU/BB/2023/06, RPCAU/BB/2023/07, RPCAU/BB/2023/08, RPCAU/BB/2023/09, RPCAU/BB/2023/10, RPCAU/BB/2023/11 and RPCAU/BB/2023/12. Cluster IB consists of RPCAU/BB/2023/01, RPCAU/BB/2023/02 and RPCAU/BB/2023/03. Cluster IIA includes RPCAU/BB/2023/13, RPCAU/BB/2023/14, RPCAU/BB/2023/15 and finally, Cluster IIB RPCAU/BB/2023/16, RPCAU/BB/2023/17, RPCAU/BB/2023/18, RPCAU/BB/2023/19, RPCAU/BB/2023/20, RPCAU/BB/2023/21 and RPCAU/BB/2023/22, Pusa Sumeet and Pusa Udit.

Discussion

Altogether, 8 qualitative traits were studied in 22 genotypes and significant variation was observed in all the traits among the genotypes collected from various regions. The analysis of variance showed significant variation among the 22 genotypes studied (1, 14–16). The best-performing and promising genotypes were determined by comparing them with the checks. The minimum number of days taken for germination was observed in the genotype RPCAU/BB/2023/07, whereas the maximum number of days was taken by RPCAU/BB/2023/11 and RPCAU/BB/2023/18, which was in agreement with previous observations (1, 14). The tallest plant among the tested genotypes was recorded in RPCAU/BB/2023/11, followed by RPCAU/BB/2023/10, while the shortest plants were observed in RPCAU/BB/2023/15. The increase in height might be due to the genotype's superior genetic capacity for internodal elongation and vegetative vigour and similar findings were also reported in previous study (1). The minimum stems per plant was recorded in RPCAU/BB/2023/03, while the

**Fig. 1.** Clustering pattern of 22 genotypes of broad bean along with checks.

maximum was recorded in RPCAU/BB/2023/07. The results obtained were consistent with previous findings (14, 17). The highest number of leaflets per leaf was recorded in RPCAU/BB/2023/13, whereas the lowest number of leaflets per leaf was observed in RPCAU/BB/2023/21. The maximum number of leaflets is primarily attributed to the genotype's inherent genetic architecture and prolonged vegetative growth phase and is in agreement with previous findings (14, 17).

The minimum node to first flowering was recorded in the line RPCAU/BB/2023/17, followed by RPCAU/BB/2023/16, while the maximum was observed in RPCAU/BB/2023/15, which was similar to previous findings (18). The minimum number of days taken for first flowering was recorded in RPCAU/BB/2023/13, followed by RPCAU/BB/2023/18, whereas the maximum number of days taken by RPCAU/BB/2023/17, which conformed with previous reports (14, 19, 20). The minimum number of days taken for 50 % flowering was recorded in RPCAU/BB/2023/13, followed by RPCAU/BB/2023/12, while the maximum was observed in RPCAU/BB/2023/20. These findings were similar to previous reports (14, 19, 20). The minimum number of days taken for first pod formation was recorded in RPCAU/BB/2023/13, followed by RPCAU/BB/2023/04, which was comparable to previous findings (14, 17).

The longest pod length was observed in the line RPCAU/BB/2023/12, followed by RPCAU/BB/2023/02, whereas the shortest pod length was recorded in RPCAU/BB/2023/18. The results were supported by previous findings (1, 14, 21, 22). The shortest pod width was recorded in RPCAU/BB/2023/22, while the longest was observed in RPCAU/BB/2023/02 and similar trends were reported previously (1, 14, 22). The highest pod of weight was recorded in the line RPCAU/BB/2023/13, whereas the lowest were observed in RPCAU/BB/2023/17. The results agreed with the findings (16, 22). The highest numbers of pods per plant were recorded in the genotype RPCAU/BB/2023/19 and the results were similar to previous findings (1, 14, 22). The minimum number of days to full pod formation is an ideal criterion for vegetable consumption; the least number of days was recorded in RPCAU/BB/2023/09, followed by RPCAU/BB/2023/01, which was in agreement with previous reports (15, 22).

The highest number of seeds per pod were recorded in RPCAU/BB/2023/08, whereas the minimum number of seeds was recorded in RPCAU/BB/2023/03. The findings were in line with previous reports (1, 14, 22). The highest seed weight was observed in RPCAU/BB/2023/02, followed by RPCAU/BB/2023/03, whereas the lowest seed weight was recorded in RPCAU/BB/2023/18 (22.50), with an average performance of 58.84. Similar findings were also reported previously (14, 21, 22). The fresh pod yield per plant was minimum in the line RPCAU/BB/2023/18, while the maximum yield was observed in RPCAU/BB/2023/11 and similar outcomes were also reported previously (1, 14, 22).

A total of 82 alleles were obtained, of which 72 were shared alleles and 10 unique allelic variants generated as amplified PCR products using 18 primers. The highest number of shared alleles per locus was recorded in primer M16, whereas the highest number of unique alleles was observed with primer M58. The PIC values ranged from 0.24 (M17) to 0.84 (M16 and M25). Two major clusters were obtained, which were further divided into 2 sub-clusters each. The results obtained were similar to previous findings (4, 7, 23, 24).

Conclusion

The analysis of variance showed significant differences among the 22 genotypes that were studied during the research program, indicating that there is a wide variation of desirable traits. Therefore, attention should be concentrated on traits that contribute to yield when selecting enhanced yield per plant, as well as in identifying suitable parental genotypes for breeding. The mean performance for the 16 traits revealed that, in comparison with the check variety, the genotypes RPCAU/BB/2023/11 and RPCAU/BB/2023/13 were found to be better in terms of yields and their associated traits, which can be determined for further use in crop improvement. The genomic DNA was extracted through the cetyl trimethyl ammonium bromide (CTAB) method and was amplified with 18 simple sequence repeat (SSR) markers using the polymerase chain reaction (PCR). The polymorphic information content (PIC) lies between 0.24 (M17) to 0.84 (M16 and M25). So, this will help the breeders identify and conserve diverse genetic resources that may be crucial for future breeding programs. The SSR markers such as M16, M25, M41 and M58 obtained high PIC values, indicating an elevated level of polymorphism, suggesting that these markers are highly informative for differentiating broad bean genotypes. Prioritising genotypes such as RPCAU/BB/2023/11 and RPCAU/BB/2023/13 provides a tangible benefit to farmers owing to their vigorous growth and consistent yield performance. Replacing lower-yielding local landraces with these high-potential lines establishes a direct route to improving farm-level profitability while securing a reliable source of vegetable protein for the region.

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Authors' contributions

LM, MP, A and N¹ carried out the laboratory analysis and initial drafting of the manuscript. LM, N¹, RKY and RA undertook material preparation, conduct of the experiment, data collection, data analysis and manuscript preparation. UK, N¹, RK, LR, N² and RKY provided overall supervision of the experiment and contributed significantly to the review and editing of the manuscript. All authors have read and approved the final manuscript. [N¹ – Neeraj and N² – Nilanjaya].

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interest to declare.

Ethical issues: None

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