



## RESEARCH ARTICLE

# Studies on genetic diversity and variability in cluster bean [*Cyamopsis tetragonoloba* (L.) Taub.] genotypes through morphological and molecular characterization

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### Abstract

The present study was carried out at the Horticulture Research Farm-I of the Department of Applied Plant Science (Horticulture), Babasaheb Bhimrao Ambedkar University, Lucknow, Uttar Pradesh, to assess genetic diversity and variability among thirty cluster bean genotypes from Rajasthan and Punjab, India, using 21 ISSR markers. A total of 263 fragments were amplified, with 204 (77.57%) being polymorphic and 59 (22.43%) monomorphic. The number of polymorphic fragments per primer ranged from four (UBC-854) to fifteen (ISSR-5 and IS-7), with an average of 9.71. Polymorphism percentage varied from 58.33% (UBC-856) to 100% (UBC-820 and ISSR-8), with an average of 77.06%. The UPGMA tree constructed using Jaccard's similarity coefficient revealed genetic relationships among the genotypes. Genetic diversity parameters, including an observed and effective number of alleles, Nei's genetic diversity, and Shannon's index, were 1.781, 1.462, 0.267, and 0.398, respectively. The total genotype diversity (Ht) was 0.2639, while within-population diversity (Hs) was 0.253. The mean coefficient of gene differentiation (Gst) was 0.041, with gene flow estimated at 11.549. Desirable genotypes identified based on both morphological and molecular characterization include IC-421834, IC-421855, IC-421828, IC-258087, IC-258092, and IC-369868. These genotypes hold the potential for breeding, gene mapping, and functional genomics to enhance cluster bean genetics.

**Keywords:** Genetic diversity, variability, cluster bean, *Cyamopsis tetragonoloba*, ISSR, germplasm.

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### Introduction

Cluster bean (*Cyamopsis tetragonoloba* L.) is an economically significant, drought-tolerant annual legume crop belonging to the family Leguminosae, mostly grown in the semi-arid regions of India. It is assumed to have evolved from the African species *C. enegalensis*, having chromosome number  $2n = 14$  (Mudgil et al., 2014). The variability of a trait describes how much that trait tends to vary in response to environmental and genetic influences. Genetic variability in a population is important for biodiversity because without variability, it becomes difficult for a population to adapt to environmental changes and therefore makes it more prone to extinction (Sousa, 2012). Information regarding the extent and pattern of genetic variation in cluster beans is limited. Therefore, urgent efforts are required to improve the yield and gum quality of cluster beans using conventional and biotechnological approaches. An assessment of genetic diversity is an important first step to achieve this goal. Previous studies on the characterization of cluster bean germplasm used phenotypic characters (Rai et al., 2012) or qualitative traits (Pathak et al., 2011). India shares 80% of world production and only 20% is produced by all other countries like Pakistan, the USA, Australia, South Africa, Sudan and Argentina (Kumar et al., 2015). Thus, the total

area under cluster bean in India is 6.82 million hectares, and production is 4.79 million tons with a productivity of 7.02 q/ha (Ministry of Agriculture & Farmers Welfare, Government of India, 2024).

Genetic divergence, a measure of biodiversity, refers to the variety of genetic traits within a species. It plays a crucial role in adaptation, allowing populations to survive environmental changes. With greater genetic variation, some individuals are more likely to possess beneficial alleles, increasing their chances of survival and reproduction. Over generations, this results in a population that is better adapted to its environment. Species with high genetic diversity have a wider pool of alleles to select from, enhancing adaptability. Studies on genetic diversity in guar have been limited, and developing genomic resources for guar is still in its early stages (Ibrahim et al., 2012; Kumar et al., 2013).

The morphological characterization provides information underlying conclusions on the genetic variability of the genotypes of the bank, identification of accessions maintained in duplicate, improvement of the data of identification and classification of accessions (Chiorato et al., 2007), and support for the regeneration and maintenance of the genetic integrity of genotypes. Characterization of genetic resources is an important component of a crop improvement program for their effective involvement in hybridization programs. The magnitude of variability and its genetic components are the most important aspects of breeding material. A great deal of information has been generated on the genetic variability of various components of cluster beans. Generally, the genotypic coefficient of variability (GCV) and phenotypic coefficient of variability (PCV) are measured to study the variability.

Genetic variation was traditionally assessed through morphological traits, but environmental influences and genotype-environment interactions limited their accuracy. Molecular markers, such as isozymes and protein electrophoresis, address these issues by directly analyzing genetic variation in DNA, providing a more reliable understanding of genetic diversity and relatedness.

The range of molecular markers that can be used on most plant germplasm is quite extensive (Mohan et al., 1997; Gupta and Varshney, 2000). Techniques vary from identifying the polymorphism in the actual DNA sequence to the use of DNA hybridization methods used to identify restriction fragment length polymorphisms (RFLPs) or the use of polymerase chain reaction (PCR)-based technology to find polymorphism using random amplified polymorphic DNA (RAPD), simple sequence repeat (SSR) or combination techniques like amplified fragment length polymorphism (AFLP). Different methods vary in cost, ease of use, data type (dominant or co-dominant markers), polymorphism detection, genetic resolution, and taxonomic utility (Karp et al., 1997). Molecular characterization is crucial in molecular

breeding and the science-based assessment of plants derived from modern biotechnology, helping to understand the introduced genetic material and its expression.

Modern molecular techniques have been developed to meet the horticulture industry's demand for genetic variation analysis, ranging from morphological characterization to DNA-based markers such as RFLP, RAPD, AFLP, and SSR (Crawford, 2000; Ferdousi Begum, 2013). Identifying and characterizing germplasm is essential for the conservation and utilization of plant genetic resources (Suvakanta-Barik, 2006). Molecular markers are superior for germplasm characterization and determining genetic relationships as they detect more polymorphisms than morphological or protein-based markers (Tanksley et al., 1989). Molecular characterization aids in understanding breeding behavior, reproductive success, gene flow, and the phylogeny, taxonomy, domestication, and evolution of plants (Nwakanma, 2003). Research on germplasm characterization and molecular analysis is crucial for improving plant resources, as demonstrated by selecting superior cluster bean varieties using both morphological and DNA-based methods.

## Materials and Methods

### *Experimental description*

In the present investigation thirty genotypes of cluster bean were carried out. Seeds of the genotypes were collected from the two places (especially, the National Bureau of Plant Genetic Resources, Regional Station, Jodhpur, India and Punjab Agricultural University, Ludhiana, India) and maintained at the Horticulture Research Farm-I of the Department of Applied Plant Science (Horticulture), Babasaheb Bhimrao Ambedkar University, Vidya-Vihar, Rae Bareilly Road, Lucknow respectively.

### *Experimental design*

The experimental field was laid out in a randomized block design (RBD) with thirty genotypes, replicated three times during the 2012-13 and 2013-14 growing seasons. Each block was further subdivided into 30 unit plots. The size of each unit plot was 2.70 × 1.20 m, with a row-to-row spacing of 45 cm and a plant-to-plant spacing of 30 cm.

### *Analysis of genetic divergence*

Genetic divergence was estimated by statistically analyzing the data on head and quality characters using Mahalanobis  $D^2$  statistics (1936) following the method proposed by Rao in 1952. Genotypes were grouped into a number of clusters.  $D^2$  is treated as the square of the generalized distance, according to the method described by Tocher (Rao, 1952). Finally, the statistical analysis for each observed character was conducted using MS Excel, OPSTAT (Sheoran et al., 1998), and IBM SPSS 20 software.

### **Molecular analysis**

Molecular biology grade chemicals used in this study were sourced from B. R. Biochem Life Sciences Pvt. Ltd., New Delhi, India, and the PCR Master Mix was obtained from Takara Biotechnology (Dalian) Co., Ltd. Other reagents for PCR analysis, including Taq polymerase, deoxyribonucleotides, assay buffer, MgCl<sub>2</sub>, and agarose, were also sourced from B. R. Biochem Life Sciences Pvt. Ltd. Primers were purchased from SBS Genentech, China. Sterile double-distilled water was used for all molecular experiments. The standard extraction buffer contained 2.5% CTAB, 100 mM Tris-HCl (pH 8), 20 mM EDTA (pH 8), 7 M NaCl, 0.5% Polyvinylpyrrolidone (PVP), and 0.2% β-mercaptoethanol (added just before use). Phenol: chloroform: iso-amyl alcohol (25:24:1), 70% alcohol, and TE buffer (1 mM Tris, pH 8.0, 1 mM EDTA, pH 8.0) were also used.

DNA was extracted from juvenile leaves (2 g) of two-week-old seedlings using the CTAB method (Doyle and Doyle, 1990). The leaves were washed, dried, and ground in liquid nitrogen. A wash buffer (0.5% PVP) was then applied, and the mixture was centrifuged. The supernatant was discarded, and the pellet was washed with 70% ethanol, air-dried, and resuspended in TE buffer. The extracted DNA was stored at -20°C for future use.

### **Analysis of molecular genetic variance (AMOVA)**

AMOVA was used to analyze variation among and within the populations. The distribution of molecular genetic variation among and within the thirty genotypes of cluster bean was estimated by AMOVA. It revealed that molecular variances were 8% of the total variance was among the subpopulations, while 92% was among individuals within the populations respectively. The same trend was observed when the AMOVA estimated based on one cluster bean types in germplasm set. The estimated variance based on ISSR marker data was 0.608 (among the population) and 6.783 (within the population). The average estimated variance was 3.695.

## **Results and Discussion**

### **Diversity and variability on the basis of morphological characters**

The genotypic and phenotypic coefficient of variation (GCV and PCV) for 11 growth characters and 8 yield and yield attributing characters revealed that all the horticultural parameters had significant range of phenotypic and genotypic coefficient of variation are presented in Table 1. In morphological and yield contributing characters, the highest phenotypic coefficient of variation was observed for number of pods/cluster (58.05) followed by number of clusters/plant (40.47) and lowest for germination % (4.46%) followed by days to maturity (3.41). The contribution of various characters towards genetic divergence is given in Table 2. Pod yield (q/ha) (20.66%) contributed maximum to

the genetic diversity among the genotypes followed by 100-seed weight (g) (20.49%), pod breadth (cm) (16.07%), number of pods/cluster (12.33%), number of pods per plant (8.42%), number of seeds/pod (6.04%) and number of clusters/plant (5.87). While there was lowest contribution found from plant height (cm) (2.47%), pod yield/plot (kg) (2.37%), pod yield/plant (g) (1.96%) and number of reproductive branches/plant (1.87%).

The genotypes were grouped into different clusters following Tocher's method as described by Rao (1952). By adopting Tocher's method, the thirty genotypes were grouped into five clusters by treating estimated D<sup>2</sup> values as the square of the generalized distance. The distribution pattern of entries into various clusters is given in the Table 3. Cluster I was the largest cluster having 8 genotypes followed by cluster II and cluster IV with six genotypes each. The computed D<sup>2</sup> values for 30 genotypes had wide range showing high genetic divergence among the genotypes (Table 3). Among the 5 clusters, cluster I with 8 genotypes followed by cluster II and cluster IV with 6 genotypes each showed maximum intra-cluster diversity. The maximum intra-cluster distance was found in cluster V (D<sup>2</sup>=6.625) closely followed by cluster II (3.813) and cluster III (3.526). Based on distance between clusters (inter-cluster), the maximum divergence was observed between cluster I and cluster II (D<sup>2</sup>=8.235), followed by cluster II and cluster IV (D<sup>2</sup>=6.552) and the least inter-cluster distance was found between cluster IV and cluster V (D<sup>2</sup>=3.773). Maximum amount of heterosis is generally accepted in cross combinations involving the parents belonging to most divergent clusters.

### **Diversity and variability on the basis of molecular characters**

Genetic diversity among thirty genotypes of cluster bean analyzed on following basis describe in this study. The primers UBC-841, UBC-855, ISSR-5 and ISSR-8 produced highest bands as compared to UBC-856 and UBC-829-11 primers produce lowest bands. The primers IS-5 and UBC-814-11 produced 13 and 11 bands respectively. The primer ISSR-2 produced unclear bands; the primer ISSR-9 did not produce any band. There are 21 primers used and produced a total of 263 bands out of which 204 were polymorphic and 59 were monomorphic. The highest bands were observed in genotypes namely, IC-258087, IC-258092, IC-28272, IC-311440, IC-311441, IC-369789 and IC-369868 and the lowest band were observed in genotypes IC-421809, IC-421812 and IC-421815. Three unique bands were observed in genotypes number IC-370490 with 2 bands in primer UBC-856 of about 320 bp and 350 bp and a single band with primer UBC-868 of about 275 bp (Table 4). The genotypes from the same geographical region were also grouped into different clusters. The different genotypes may have

**Table 1:** Estimates of phenotypic and genotypic coefficients of variability, heritability, genetic advance and genetic advance in percent of mean for different characters in thirty genotypes of cluster bean

Characters	General Mean	SEM ±	Range		Coefficients of variability			$h^2_{bs}$ (%)	GA	GAPM (%)
			Min.	Max.	CV (%)	PCV (%)	GCV (%)			
Plant height (cm)	80.24	3.07	55.71	107.69	6.63	17.42	16.11	85.50	24.62	30.68
Germination (%)	91.64	2.10	87.24	95.94	3.98	4.46	2.00	20.20	1.70	1.85
Days taken for first flowering	22.40	0.831	20.46	23.83	6.42	6.91	2.54	13.50	0.43	1.91
Days taken for 50% flowering	33.55	0.813	31.40	36.34	4.20	5.18	3.03	34.20	1.22	3.63
Number of reproductive branches/plant	5.86	0.365	4.13	7.75	10.80	19.81	16.61	70.20	1.68	28.66
Pod breadth (cm)	3.75	0.269	3.29	4.22	12.43	13.85	6.09	19.40	0.21	5.60
Pod length (cm)	5.71	0.336	4.57	6.66	10.20	13.34	8.60	41.50	0.65	11.38
Pod width (cm)	5.95	0.433	5.68	6.34	12.60	12.93	2.88	5.00	0.08	1.34
Number of pods/plant	56.36	3.16	42.47	73.74	9.73	15.33	11.84	59.70	10.62	18.84
Number of pods /cluster	7.74	0.452	4.33	20.22	12.13	58.05	56.77	95.60	8.85	114.34
Number of clusters/plant	11.80	0.498	6.83	29.33	7.31	40.47	39.80	96.70	9.52	80.67
Number of branches /plant at maturity	8.41	0.217	7.25	10.17	4.47	8.77	7.54	73.90	1.12	13.31
Number of seeds /pod	6.76	0.241	5.95	7.52	6.19	9.11	6.68	53.80	0.68	10.05
Pod yield /plant (g)	96.32	1.96	79.23	121.49	3.53	13.21	12.72	92.80	24.33	25.25
Pod yield /plot (kg)	0.57	0.032	0.39	0.76	9.89	18.20	15.27	70.40	0.15	26.31
100- Seed weight (g)	3.63	0.167	3.00	4.33	7.96	11.96	8.92	55.60	0.50	13.77
Seed yield /plant (g)	6.69	0.302	4.79	8.64	7.81	15.91	13.86	75.90	1.66	24.81
Days to maturity	92.53	0.738	86.42	97.27	1.38	3.41	3.12	83.60	5.44	5.87
Pod yield (q/ha)	126.93	2.72	119.40	142.23	3.72	5.80	4.45	58.80	8.92	7.02

PCV: Phenotypic coefficient of variability; GCV: Genotypic coefficient of variability;  $h^2_{bs}$ : Heritability in broad sense; GA: Genetic advance in percent of mean

whole or partial common pedigrees and may have been subjected to the same selection during their breeding but are still distinguishable from each other on the basis of RAPD profiles. Lavanya et al. (2008) have reported lack of correlation between geographic and genetic diversity in other legumes. RAPD markers have been used for the identification of cultivars and the genetic relationships among cultivars of other leguminous crops including *Phaseolus vulgaris* (Skroch et al., 1992) and *V. angularis* (Yee et al., 1999) and *V. radiata* (Lavanya et al., 2008). The present study has revealed that there is wide genetic base in *C. tetragonoloba* genotypes for crop improvement. The gel image of this character has been described in Figure 1.

### Analysis of molecular genetics variance (AMOVA)

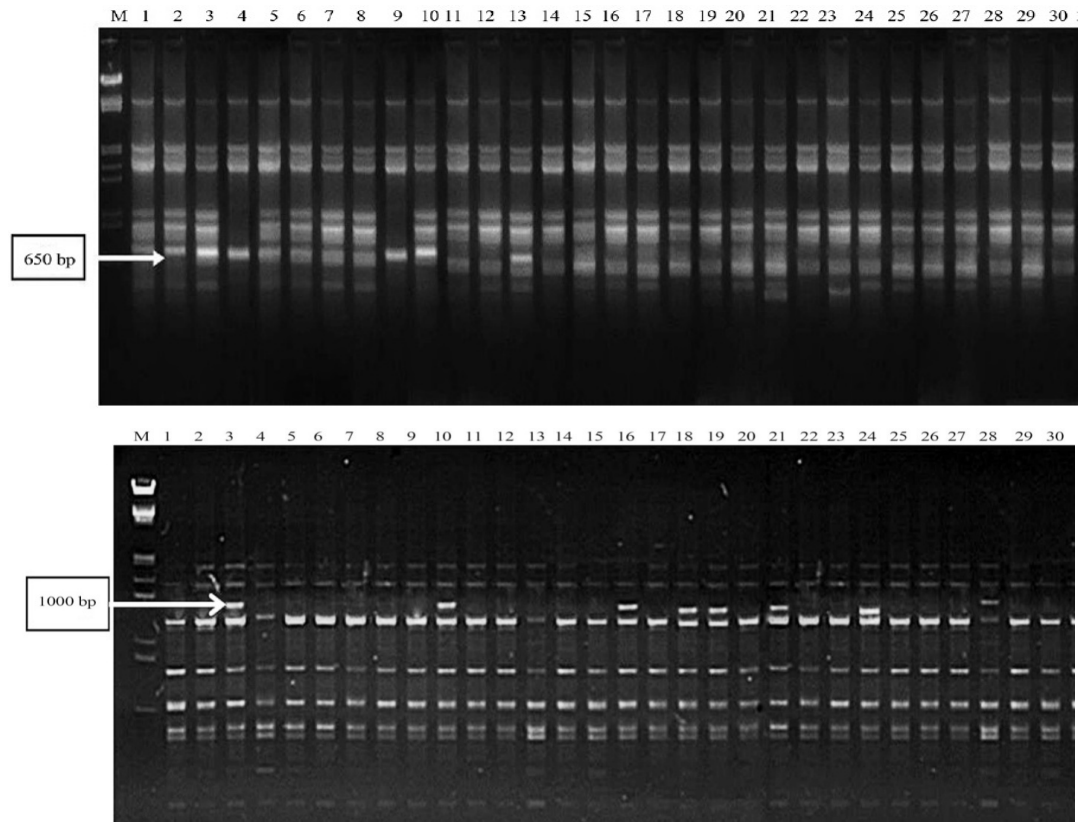
AMOVA was used to analyze genetic variation among and within thirty cluster bean genotypes. The results showed that 8% of the total variance was between subpopulations, while 92% was within individuals of the populations. A similar trend was observed for the germplasm set based on cluster bean types. For ISSR marker data, the estimated variance was 0.608 among populations and 6.783 within populations, with an average variance of 3.695.

## Discussion

### Morphological characters

The success of a breeding program depends on selecting the right parents, with divergent crosses often yielding useful progenies. Mahalanobis' (1928)  $D^2$  analysis is an effective tool for assessing genetic divergence, helping to choose optimal parents for recombination breeding. Quantifying genetic diversity within and between germplasm groups is crucial for achieving higher heterosis and useful recombinants.  $D^2$  statistics considers multiple parameters, providing a more comprehensive approach than relying on morphological, eco-geographical, or phylogenetic indices. Among the 19 characters studied, pod yield per hectare was the most important contributor to genetic divergence, followed by 100-seed weight, pod breadth, number of pods/clusters, pods per plant, seeds per pod, and clusters per plant (5.87). These findings align with Singh et al. (2003) and Henry et al. (1984), while the contribution of pods per cluster and pods per plant agrees with Gipson and Balakrishnan (1992), and plant height aligns with Hanchinamani (2004).

The phenotypic and genotypic coefficients of variation (PCV and GCV) reflect the extent of variability in a population for morphological and yield traits. In this study, the highest phenotypic coefficient of variation was observed for the number of pods per cluster (58.05), followed by the number of clusters per plant (40.47), and the lowest for days to maturity (3.41) and germination percentage (4.46%). The genotypic coefficient of variation was highest for the number of pods per cluster (56.77) and number of clusters



**Figure 1:** ISSR profiling of thirty cluster bean genotypes with primer ISSR-5 and ISSR-8 digested at 650 bp and 1000 bp respectively

per plant (39.80), with the lowest values for germination percentage (2.00%) and days to first flowering (2.54%). These results are consistent with the findings of Singh et al. (1999).

In this study, the phenotypic coefficient of variation (PCV) was higher than the genotypic coefficient of variation (GCV), indicating that the observed variation was influenced by both genotype and environment. Traits such as pod yield (q/ha), pod yield per plant, germination percentage, plant height, and seed yield per plant showed a high range of variation, suggesting they have good potential for improvement through selection. Characters like pod yield per plant, pod yield per plot, and pod yield (q/ha) exhibited moderate GCV and PCV values, indicating they can be effectively improved through selection. The estimates of GCV and PCV for traits like 100-seed weight, pod yield (q/ha), plant height, and pod yield per plant highlight the importance of these traits in the improvement program.

GCV alone helps assess the amount of heritable variation in a trait. The heritability estimate indicates the degree to which a trait is passed from parent to offspring. High heritability suggests that environmental factors have less influence on the trait's expression, making it more suitable for improvement through simple selection methods. However, heritability estimates, combined with genetic advance, provide a clearer picture of the potential gain from selection. Therefore, both heritability and genetic advance

**Table 2:** Contribution of various characters towards genetic divergence in thirty genotypes of cluster bean

S. No.	Characters	Contribution (%)
1.	Plant height (cm)	2.47
2.	Germination (%)	0.51
3.	Days taken for first flowering	0.43
4.	Days taken for 50% flowering	0.17
5.	Number of reproductive branches/plant	1.87
6.	Pod breadth (cm)	16.07
7.	Pod length (cm)	0.43
8.	Pod width (cm)	0.09
9.	Number of pods/plant	8.42
10.	Number of pods /cluster	12.33
11.	Number of clusters /plant	5.87
12.	Number of branches /plant at maturity	0.77
13.	Number of seed /pod	6.04
14.	Pod yield /plant (g)	1.96
15.	Pod yield /plot (kg)	1.45
16.	100- Seed weight (g)	20.49
17.	Seed yield /plant (g)	0.00
18.	Days to maturity	0.00
19.	Pod yield (q/ha)	20.66

are essential for determining the scope of improvement in various traits through selection.

Genotypic and phenotypic correlations were calculated to assess the association between component characters and pod yield (q/ha), while path coefficient analysis was

**Table 3:** Average intra and inter cluster D<sup>2</sup> and D values in thirty genotypes of cluster bean

S. No.	Cluster distances	Cluster I	Cluster II	Cluster III	Cluster IV	Cluster V
1.	Cluster I	3.400	8.235	5.327	6.552	6.625
2.	Cluster II		3.813	4.218	5.263	6.428
3.	Cluster III			3.526	4.525	5.348
4.	Cluster IV				3.464	3.773
5.	Cluster V					5.243

**Table 4:** Details of ISSR primers showing number of band and percentage of polymorphism generated from thirty genotypes of cluster bean

S. No.	Primer Name	Sequence (5'-3')	Number of amplified fragments	Polymorphic fragments	Polymorphism percentage (%)	Monomorphic fragment	Monomorphism percentage (%)
1.	UBC-808	AGAGAGAGAGAGAGGC	12	9	75.00	3	25.00
2.	UBC-818	CACACACACACACAG	14	10	71.43	4	28.57
3.	UBC-820	GTGTGTGTGTGTGTC	7	7	100.00	0	0.00
4.	UBC-854	TCTCTCTCTCTCTCRG	6	4	66.67	2	33.33
5.	UBC-856	ACACACACACACACYA	12	7	58.33	5	41.66
6.	UBC-868	GAAGAAGAAGAAGAAGAA	15	12	80.00	3	20.00
7.	UBC-879	CTTCACTTCACTTCA	14	10	71.43	4	28.57
8.	ISSR-1	GAGAGAGAGAGAGAC	14	12	85.71	2	14.28
9.	ISSR-2	CACACACACACACAC	10	7	70.00	3	30.00
10.	ISSR-5	CACACACACACAGG	15	13	86.67	2	13.33
11.	ISSR-8	GAGAGAGAGAGACC	15	15	100.00	0	0.00
12.	ISSR-9	GTGTGTGTGTGTCC	10	8	80.00	2	20.00
13.	IS-5	AGAGAGAGAGAGAGGT	13	9	69.23	4	30.76
14.	IS-7	GAGAGAGAGAGAGAT	16	15	93.75	1	6.25
15.	IS-14	GTGTGTGTGTGTGTC	10	7	70.00	3	30.00
16.	IS-25	AGCAGCAGCAGCAGCAGC	14	9	64.29	5	35.71
17.	UBC 814-11	CTCTCTCTCTCTCAT	11	8	72.73	3	27.27
18.	UBC 826-11	ACACACACACACACC	13	9	69.23	4	30.76
19.	UBC 829-11	TGTGTGTGTGTGTGT	8	6	75.00	2	25.00
20.	UBC 841	GAGAGAGAGAGAGAYC	17	14	82.35	3	17.64
21.	UBC 855	ACACACACACACACYT	17	13	76.47	4	23.52
Total			263.00	204.00	1618.29	59.00	481.65
General mean			12.52	9.71	77.06	2.80	22.93

used to understand the nature of these associations (Dewey and Lu, 1959). The analysis of variance revealed highly significant differences for all characters studied. Although breeding programs rely on variability, absolute variability in traits is not the sole factor in determining which traits exhibit the highest variability. Therefore, the available variability in morphological and yield-contributing characters was quantified to identify desirable genotypes based on performance. This helps select promising donors

for hybridization programs, aiming to obtain useful recombinants and create additional genetic variability.

#### **Molecular characters**

Genetic diversity among thirty genotypes of cluster bean analyzed on following basis describe in this study. The primers UBC-841, UBC-855, ISSR-5 and ISSR-8 produced highest bands as compared to UBC-856 and UBC-829-11 primers produce lowest bands (Table 4). The primers IS-5

and UBC-814-11 produced 13 and 11 bands respectively. The primer ISSR-2 produced unclear bands; the primer ISSR-9 did not produce any band. There are 21 primers used and produced a total of 263 bands out of which 204 were polymorphic and 59 were monomorphic. The highest bands were observed in genotypes namely, IC-258087, IC-258092, IC-28272, IC-311440, IC-311441, IC-369789 and IC-369868 and the lowest band were observed in genotypes IC-421809, IC-421812 and IC-421815. Three unique bands were observed in genotypes number IC-370490 with 2 bands in primer UBC-856 of about 320 bp and 350bp and a single band with primer UBC-868 of about 275 bp. RAPD and ISSR marker generated good diversity for the present set of cluster bean genotypes. Earlier studies also showed a large genetic variation among different genotypes of cluster bean using the RAPD (Punia et al., 2009; Pathak et al., 2010).

AMOVA was used to analyze genetic variation among and within populations of thirty cluster bean genotypes. The results revealed that 8% of the total molecular variance was attributed to differences among subpopulations, while 92% was observed within individuals of the populations. Similar trends were observed when AMOVA was estimated for a single cluster bean type in the germplasm. The variance estimated from ISSR marker data was 0.608 among the populations and 6.783 within the populations, with an average variance of 3.695. These findings align with the results of Aswathnarayana et al. (2013).

## Conclusion

This study concluded that morphological trait, such as number of pods/plant, pod yield/plant, seed yield/plant, pod yield (q/ha), number of seeds/pod, and 100-seed weight, are reliable for varietal characterization in cluster bean. Based on these traits, superior genotypes identified for higher yield were IC-421834, IC-421855, IC-421828, HG-365, IC-421809, and IC-421806. Additionally, ISSR markers proved effective in assessing genetic diversity and variability, providing a foundation for efficient germplasm utilization, conservation, and management. The identified germplasm will support functional genomics, gene mapping, and breeding programs to enhance the genetic potential of cluster bean. Molecular markers classified the genotypes into five distinct clusters, with the most promising genotypes being IC-258087, IC-258092, IC-28272, IC-311440, IC-311441, IC-369789, and IC-369868.

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## सारांश

वर्तमान अध्ययन राजस्थान और पंजाब के तीस क्लस्टर बीन जीनोटाइप के बीच आनुवंशिक विविधता और परिवर्तनशीलता का आकलन करने के लिए बाबासाहेब भीमराव अंबेडकर विश्वविद्यालय, लखनऊ, उत्तर प्रदेश के एप्लाइड प्लांट साइंस (बागवानी) विभाग के बागवानी अनुसंधान फार्म- I में किया गया था। भारत, 21 आईएसएसआर मार्करों का उपयोग कर रहा है। कुल 263 टुकड़े प्रवर्धित किए गए, जिनमें 204 (77.57%) बहुरूपी और 59 (22.43%) मोनोमोर्फिक थे। प्रति प्राइमर बहुरूपी टुकड़ों की संख्या चार (यूबीसी-854) से पंद्रह (आईएसएसआर-5 और आईएस-7) तक थी, जिसका औसत 9.71 था। बहुरूपता प्रतिशत 58.33% (यूबीसी-856) से 100% (यूबीसी-820 और आईएसएसआर-8) तक भिन्न था, औसत 77.06% के साथ। जैकार्ड के समानता गुणांक का उपयोग करके बनाए गए यूपीजीएम पेड़ ने जीनोटाइप के बीच आनुवंशिक संबंधों का खुलासा किया। एलील्स की देखी गई और प्रभावी संख्या, नेई की आनुवंशिक विविधता और शैनन के सूचकांक सहित आनुवंशिक विविधता पैरामीटर क्रमशः 1.781, 1.462, 0.267 और 0.398 थे। कुल जीनोटाइप विविधता (एचटी) 0.2639 थी, जबकि जनसंख्या के भीतर विविधता (एचएस) 0.253 थी। जीन विभेदन (जीएसटी) का औसत गुणांक 0.041 था, जीन प्रवाह 11.549 अनुमानित था। रूपात्मक और आणविक लक्षण वर्णन के आधार पर पहचाने जाने वाले वांछनीय जीनोटाइप में IC-421834, IC-421855, IC-421828, IC-258087, IC-258092 और IC-369868 शामिल हैं। ये जीनोटाइप क्लस्टर बीन आनुवंशिकी को बढ़ाने के लिए प्रजनन, जीन मैपिंग और कार्यात्मक जीनोमिक्स की क्षमता रखते हैं।