

Biotechnological Interventions in Unravelling the Genetic Treasure of an Orphan Crop, Cluster Bean (*Cyamopsis tetragonoloba* L.)

Priti .^{1, 2}, Sumit Jangra³, Vrantika Chaudhary¹, Rizwana Rehsawla¹, Aditi Taneja¹, Jugpreet Singh³, Ram C. Yadav¹, Neelam R. Yadav¹

1. Department of Molecular Biology, Biotechnology, and Bioinformatics, CCS Haryana Agricultural University, Hisar, IND

2. Department of Biotechnology, DAV University, Jalandhar, IND

3. Tropical Research and Education Center, University of Florida, Homestead, USA

Received: December 28, 2025 | Review began: December 31, 2025 | Review ended: May 06, 2026 | Published: May 20, 2026

© Copyright 2026

This is an open access article distributed under the terms of the Creative Commons Attribution License CC-BY 4.0., which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Abstract

Cluster bean [*Cyamopsis tetragonoloba* (L.) Taub.], also known as guar, is an underutilized crop grown in arid and semi-arid areas. It is mainly grown for vegetable and guar gum production. Guar gum is a natural, non-toxic polymer with great industrial potential. The foremost industrial targets are mining, oil and gas well fracturing, paper, food, pharmaceuticals, textiles, cosmetics, and explosives. Despite having significant industrial importance, guar research has received less attention than other major legumes. However, with the increase in industrial demand in recent years, progress has been made in better understanding of cluster bean genetics. This review summarizes the recent progress in molecular marker development, quantitative trait locus mapping, omics-based approaches, and functional genomics in cluster bean. Although large numbers of simple sequence repeats and single-nucleotide polymorphisms have been identified, their application remains largely restricted to diversity analysis, with limited integration into breeding programs. Genome-wide association studies and quantitative trait locus mapping efforts are still scarce, and many reported associations lack validation across populations, constraining the identification of stable loci for key traits such as gum content, stress tolerance, and yield. Omics studies have provided insights into galactomannan biosynthesis and stress-responsive pathways; however, most findings lack functional validation and practical deployment. Recent developments in RNA interference and CRISPR/Cas9-based genome editing provide new opportunities for precise functional validation and targeted crop improvement. Therefore, bridging the gap between the genomic discoveries and their implementation into breeding strategies is key to developing improved cluster bean cultivars that can meet the industry demands.

Categories: Agricultural and food economics, Field & Horticultural crops sustainable production, Trait genetics & breeding of crops

Keywords: cluster bean, molecular markers, omics, mirna, biotic stress, abiotic stress, guar gum

Introduction And Background

Cluster bean [*Cyamopsis tetragonoloba* (L.) Taub.], commonly referred to as guar, is an important leguminous crop belonging to the genus *Cyamopsis* with chromosome number $2n = 14$ [1]. Genus *Cyamopsis* comprises three species, namely, *C. tetragonoloba*, *C. senegalensis*, and *C. serrata*, where *C. tetragonoloba* is a domesticated form of the African wild species *C. senegalensis* [2]. Guar is well adapted to low humidity and water-scarce conditions, and demands minimal agricultural inputs, making it well-suited to arid environments. It is mainly cultivated in countries such as India, South Africa, Pakistan, Myanmar, Indonesia, and the United States [3].

How to cite this article:

. P, Jangra S, Chaudhary V, et al. (May 20, 2026) Biotechnological Interventions in Unravelling the Genetic Treasure of an Orphan Crop, Cluster Bean (*Cyamopsis tetragonoloba* L.). Curues J. Agri. Food Sci 2 : es44497-025-00069-2. DOI <https://doi.org/10.7759/s44497-025-00069-2>

India currently accounts for approximately 75-80% of the total world's guar production [4]. This dominance is supported by the development of 39 improved varieties between 1976 and 2023 [5]. Historically, guar is primarily grown for its utility as green manure, food, and forage. The endosperm of guar seeds produces a natural polysaccharide, commonly referred to as 'guar gum', that acts as a viscosity builder and has developed widespread applications in food processing, pharmaceuticals, oil well drilling, paper, cosmetics, regenerative medicines, textile industries, and others [2]. During FY 2024-25, India exported 453,612 metric tonnes of guar gum, valued at USD 568.97 million. Export trends over the past five years have fluctuated, with a sharp dip in FY 2020-21, followed by a peak in FY 2022-23 and a slight decline thereafter (<https://apeda.gov.in/GuarGum>). These fluctuations are largely driven by the crop's strong dependence on the global oil and gas industry, particularly its use in hydraulic fracturing, making guar demand highly cyclical and sensitive to external industrial factors. Despite this economic significance, cluster bean remains relatively underexplored at the genomic and breeding levels compared to major legume crops. The limited availability of validated molecular markers, structured mapping populations, and functionally characterized genes restrict the efficient utilization of genetic resources and slow the development of improved cultivars.

Guar production is constrained by several abiotic and biotic stresses that limit the yield and quality of important agronomic traits of the crop [6,7]. Drought stress is one of the major limiting factors, with tolerance varying among guar genotypes [8] and associated with varied levels of physiological and metabolic adjustments [9]. In addition to drought, salt stress is also a major limiting factor in guar productivity, while some genotypes are available to combat salt stress in the guar crop. Genotypes such as 'Matador' and PI268229 are reported to exhibit relatively greater salinity tolerance than other genotypes [10]; moreover, genotype 'Monument' demonstrated strong adaptation to the Mediterranean environment [11]. However, systematic screening of global germplasm for stress tolerance remains limited. Furthermore, biotic stresses, like alternaria leaf blight, dry root rot, anthracnose, bacterial leaf blight (BLB), and yellow vein mosaic virus, can elicit yield losses up to 50-70% [12-14]. Insect pests such as thrips, aphids, whiteflies, and borers further intensify yield losses [15]. Chemical control measures are being employed but remain associated with environmental risks and resistance development. The development of stress-resilient guar genotypes represents an environmentally friendly approach to managing stress in crops. Nevertheless, this objective is impeded by the limited availability of genetic diversity in cultivated guar genotypes and the absence of complete resistance in available varieties. However, genotypes such as GAUG9406 exhibit resistance to various biotic stresses of the crop, identified as an important source of resistance in breeding programs [16]. Additionally, varieties HG2-20 and HG563 displayed high seed yield and gum content, respectively, making them a valuable source for quality improvement [5,16]. However, no cultivar has demonstrated complete resistance to major stresses, which is mainly attributed to the narrow genetic base of the crop.

Expanding the genetic base through wild relatives such as *C. serrata* and *C. senegalensis* could be a promising strategy; however, their utilization is largely constrained by reproductive barriers and poor hybrid viability. Consequently, conventional breeding efforts are hindered by narrow genetic diversity, limited resistant germplasm, and complex trait inheritance [17-19].

Recent developments in molecular tools have provided new opportunities to overcome these limitations. Earlier studies relied on dominant markers such as random amplified polymorphic DNA (RAPD) and inter simple sequence repeat (ISSR) for germplasm characterization [6,20-24], whereas the use of co-dominant markers such as simple sequence repeats (SSRs) and sequence characterized amplified region (SCAR) was initially limited due to a lack of genomic resources [25-28]. Over the course of time, the availability of chloroplast genome sequences [29] and subsequent whole-genome assemblies [30,31] has considerably enriched genomic resources in this crop. These developments have enabled large-scale identification of molecular markers [SSRs, single-nucleotide polymorphism (SNPs), insertions and deletions (InDels)], construction of linkage maps, and trait association studies [31-33].

In parallel, transcriptomic approaches have facilitated the identification of genes and regulatory networks associated with stress responses and gum biosynthesis [31,34,35]. Notably, several tissue-specific miRNAs involved in gene regulation and galactomannan synthesis have been identified [34-37]. Functional genomics tools, including RNA interference (RNAi) [38,39] and CRISPR/Cas-based genome editing [40], have emerged as a powerful tool to characterize

How to cite this article:

. P, Jangra S, Chaudhary V, et al. (May 20, 2026) Biotechnological Interventions in Unravelling the Genetic Treasure of an Orphan Crop, Cluster Bean (*Cyamopsis tetragonoloba* L.). Curues J. Agri. Food Sci 2 : es44497-025-00069-2. DOI <https://doi.org/10.7759/s44497-025-00069-2>

gene function and targeted crop improvement. While RNAi has enabled functional validation of genes related to disease resistance and gum synthesis, recent advances in CRISPR-based targeting of *C. tetragonoloba phytoene desaturase* (*CtPDS*) gene have laid the foundation for precise genome modification in cluster bean [40].

Cumulatively, these advances have laid the foundation for integrating advanced molecular tools and omics in guar improvement programs; however, their practical applications in breeding programs remain limited. This review critically examines how the current knowledge on molecular markers and omics approaches in cluster bean has accelerated genetic improvement for stress tolerance, yield, and gum quality. Furthermore, we focused on how these resources helped in identifying key gaps limiting their translation into breeding applications.

Review

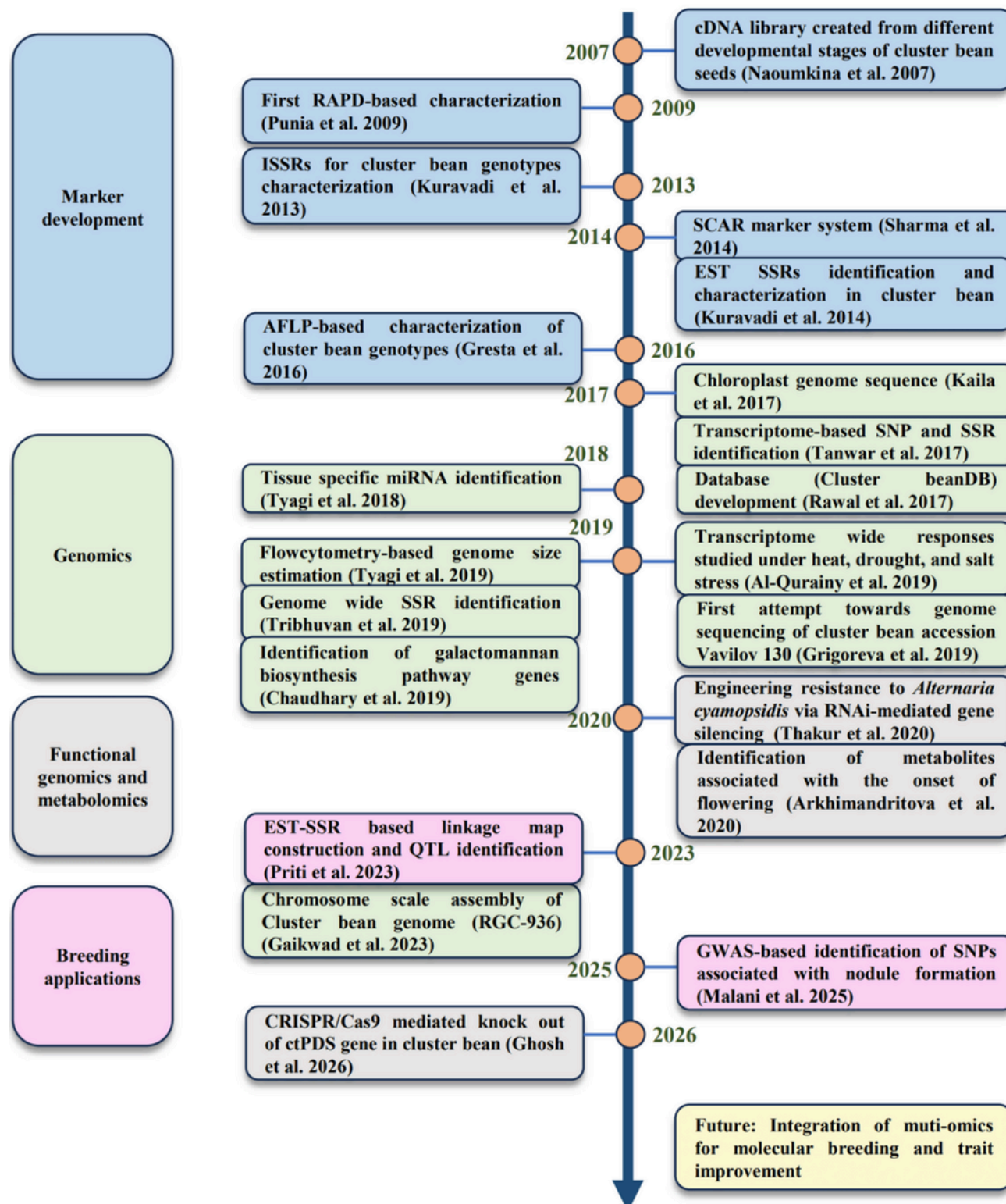
DNA-based markers

Identifying and utilizing suitable germplasm is central to crop improvement; however, reliance on morphological traits alone limits accurate assessment of genetic diversity. Moreover, it is difficult to determine the genetic background of selected germplasm solely based on the morphological features. Over the last two decades, marker development in cluster bean has evolved from low-resolution dominant markers (RAPD, ISSR) to high-density SNP-based systems enabled by next-generation sequencing. However, this transition remains incomplete, with most studies still relying on low-density markers (Figure 7) [41].

How to cite this article:

. P, Jangra S, Chaudhary V, et al. (May 20, 2026) Biotechnological Interventions in Unravelling the Genetic Treasure of an Orphan Crop, Cluster Bean (*Cyamopsis tetragonoloba* L.). Curues J. Agri. Food Sci 2 : es44497-025-00069-2. DOI <https://doi.org/10.7759/s44497-025-00069-2>

Timeline of molecular and genomic advancements in cluster bean



How to cite this article:

. P, Jangra S, Chaudhary V, et al. (May 20, 2026) Biotechnological Interventions in Unravelling the Genetic Treasure of an Orphan Crop, Cluster Bean (*Cyamopsis tetragonoloba* L.). Curues J. Agri. Food Sci 2 : es44497-025-00069-2. DOI <https://doi.org/10.7759/s44497-025-00069-2>

FIGURE 1: Timeline of key technological milestones in cluster bean research, highlighting the transition from early marker systems to advanced genomics, functional genomics, and breeding applications, including genome sequencing, omics-based trait discovery, and genome editing strategies.

AFLP, Amplified Fragment Length Polymorphism; EST-SSR, Expressed Sequence Tag-Simple Sequence Repeat; RAPD, Random Amplified Polymorphic DNA; SCAR, Sequence Characterized Amplified Region; SNP, Single-Nucleotide Polymorphism; SSR, Simple Sequence Repeat

Dominant markers

In the absence of codominant markers, research was primarily focused on developing markers that do not require prior DNA sequence information, which could further aid in comprehending different genomic regions of the cluster bean. This includes the development of dominant RAPD and ISSR markers to understand genetic diversity in the cluster bean germplasm [6,20-24,27]. RAPD markers do not require prior DNA sequence information (Table 1), and few primers can generate a sufficient diversity index within the population [22,42,43]. Pathak et al. [22] characterized 32 cluster bean genotypes procured from different regions of India, like Rajasthan, Haryana, and Gujarat, using RAPD primers. Among these 32 genotypes, 10 genotypes, viz. RGC 1038, CAZG 6, RGC 1002, RGC 1033, RGC 986, HGS 880, HGS 870, HGS 884, and RGC 1088, were highly distinct and can be used in further breeding programs.

How to cite this article:

. P, Jangra S, Chaudhary V, et al. (May 20, 2026) Biotechnological Interventions in Unravelling the Genetic Treasure of an Orphan Crop, Cluster Bean (*Cyamopsis tetragonoloba* L.). Curues J. Agri. Food Sci 2 : es44497-025-00069-2. DOI <https://doi.org/10.7759/s44497-025-00069-2>

Total number of accessions	Purpose of the study	Markers	Outcome	Reference
15 accessions	Diversity analysis	12 RAPDs	Most distinct accessions were 24293 and 24302	[6]
8 genotypes	Diversity analysis	AFLPs	Indian genotypes exhibited relatedness to American genotypes, while they were distant to African genotypes	[11]
34 genotypes	Diversity analysis	10 RAPDs	RGC1038, CAZG6, RGC1002, RGC1033, RGC986, HGS884, HGS884, and RGC1088 identified as most distinct genotypes	[20]
18 varieties	Diversity analysis	37 RAPDs	Discrete clustering of different genotypes into groups	[21]
33 varieties	Diversity analysis	5 RAPDs	Most diverse genotypes were RGC1002 and CAZG6	[22]
48 genotypes	Diversity analysis	13 RAPDs, 7 ISSRs	Clearly differentiated landraces from commercial varieties	[23]
30 genotypes	Diversity analysis	21 ISSRs	ISSR amplification protocol standardized	[24]
15 genotypes	Diversity analysis	3 RAPDs	High genetic similarity observed between AVTG-2 and IVTG-8	[42]
35 genotypes	Diversity analysis	40 RAPDs, 10 ISSRs	Substantial genetic diversity observed among genotypes	[44]
33 genotypes	Diversity analysis	20 RAPDs, 10 ISSRs	Both RAPDs and ISSRs distinguished all 35 genotypes	[45]
56 genotypes	Diversity analysis	25 RAPDs	Wild species were found to be genetically	[46]

How to cite this article:

. P, Jangra S, Chaudhary V, et al. (May 20, 2026) Biotechnological Interventions in Unravelling the Genetic Treasure of an Orphan Crop, Cluster Bean (*Cyamopsis tetragonoloba* L.). Curues J. Agri. Food Sci 2 : es44497-025-00069-2. DOI <https://doi.org/10.7759/s44497-025-00069-2>

			distinct from <i>C. tetragonoloba</i>	
12 genotypes	Diversity analysis	19 RAPDs	GAUG-0522 and GAUG-9404 identified as most distinct genotypes	[47]
104 genotypes	Diversity analysis	10 RAPDs, 10 ISSRs	Wide range of diversity observed	[48]
5 genotypes	Diversity analysis	224 EST-SSRs	SSRs differentiated three genotypes and provided a set of 187 EST-SSRs	[25]
20 genotypes	Diversity analysis	36 EST-SSRs	Differentiated genotypes with high and low gum content	[26]
31 varieties	Diversity analysis	17 SSRs	Low genetic variations observed	[27]
31 genotypes	Diversity analysis	21 ISSRs	Considerable diversity observed	[49]
Cross between HG75 and PNB	Biparental QTL mapping	83 RAPDs	One major and two minor QTLs for BLB resistance identified	[50]
34 varieties	Diversity analysis	10 ISSRs, 14 RAPDs	Higher genetic diversity was observed with in the population than between the populations	[51]
35 genotypes	Diversity analysis	20 SCARs	Genotype-specific SCARs developed	[52]
74 genotypes	Diversity analysis	25 SSRs	Wide range of diversity observed	[53]
32 genotypes	Diversity analysis	50 EST SSRs	Revealed very low genetic diversity	[54]
158 genotypes	GWAS	85 EST-SSRs	22 marker trait associations were observed	[55]
25 genotypes	Diversity analysis	11 EST SSRs	Differentiated wild species from cultivated genotypes	[56]

How to cite this article:

. P, Jangra S, Chaudhary V, et al. (May 20, 2026) Biotechnological Interventions in Unravelling the Genetic Treasure of an Orphan Crop, Cluster Bean (*Cyamopsis tetragonoloba* L.). *Curues J. Agri. Food Sci 2* : es44497-025-00069-2. DOI <https://doi.org/10.7759/s44497-025-00069-2>

HG563 × <i>C. serrata</i>	Biparental QTL mapping	177 SSRs	4 minor QTLs identified	[57]
21 genotypes	Diversity analysis	20 SSR markers	Moderate diversity observed	[58]
54 genotypes	Diversity analysis	59 SSRs	Distinguished wild relatives from <i>Cyamopsis tetragonoloba</i>	[28]
50 genotypes	Diversity analysis	21 genomic SSRs	Showed low genetic variability in guar gene pool	[59]
46 genotypes	Diversity analysis	157 nuclear SSRs	Significant diversity observed among germplasm	[17]
166 genotypes	GWAS	4,907 SNPs 327 InDels	Markers linked to important agronomic traits identified	[60]
225 genotypes	GWAS	7,000 SNPs	SNPs associated with plant height, nodule number and weight per plant, fresh and dry above ground biomass identified	[19]

TABLE 1: List of DNA-based markers employed in the characterization of cluster bean genotypes or varieties.

AFLP, Amplified Fragment Length Polymorphism; BLB, Bacterial Leaf Blight; EST-SSR, Expressed Sequence Tag-Simple Sequence Repeat; ISSR, Inter Simple Sequence Repeat; QTL, Quantitative Trait Locus; RAPD, Random Amplified Polymorphic DNA; SCARs, Sequence Characterized Amplified Regions; SNP, Single-Nucleotide Polymorphism

Moreover, genetic diversity studies of cluster bean have also employed ISSR markers in combination with RAPDs or in solitary [2,45,46,51,52]. A study by Sharma et al. [45] explored genetic diversity among cluster bean genotypes RGC-936, RGC-1002, RGC-1003, RGC-1017, and RGC-1031 using 15 RAPD primers, and the markers exhibited 66% average polymorphism. Kuravadi et al. [23] introduced ISSR markers along with RAPDs for the first time in characterizing 29 landraces and 19 commercial genotypes of cluster beans. They used a total of 13 RAPD markers and 7 ISSR markers. The average percent polymorphism by RAPD and ISSR markers was found to be 87.63% and 77.82%, respectively. UPGMA tree was constructed using RAPD and RAPD+ISSR markers, reflecting the separate clustering of landraces from commercial varieties. Ansari et al. [48] investigated the diversity profiles among 104 cluster bean genotypes collected from different areas of India. They screened 60 RAPD and 50 ISSR markers for molecular diversity analysis among genotypes. In the results, percent polymorphism observed among genotypes was 87% and 97% by RAPD and ISSR markers, respectively. Cumulative molecular data was further used for cluster analysis and revealed six major clusters, suggesting the many genetic lineages present in the genotypes. The information generated from their study can be further used in selection and improvement programs. Although dominant markers have limitations related to reproducibility and dominance,

How to cite this article:

. P, Jangra S, Chaudhary V, et al. (May 20, 2026) Biotechnological Interventions in Unravelling the Genetic Treasure of an Orphan Crop, Cluster Bean (*Cyamopsis tetragonoloba* L.). *Curues J. Agri. Food Sci 2* : es44497-025-00069-2. DOI <https://doi.org/10.7759/s44497-025-00069-2>

they continue to be used in cluster bean research primarily due to their low cost and independence from prior sequence information. These features make dominant markers particularly useful in under-resourced systems and in crops like cluster bean, where genomic resources were historically limited. Additionally, they have enabled rapid preliminary assessment of genetic diversity and screening of large germplasm collections.

Besides RAPD and ISSRs, amplified fragment length polymorphism (AFLP) has also been used for germplasm characterization in cluster bean. Gresta et al. [11] used 11 AFLP markers to deduce the genetic similarity between eight cluster bean genotypes from India, South Africa, and the United States [11]. Their result reflected more similarity between the cluster bean genotypes from India and the United States. However, Indian genotypes appeared to be more diverse than the South African genotypes. Dominant markers such as RAPD, ISSR, and AFLP have significantly contributed towards the improvement in the assessment of genetic diversity among cluster bean genotypes over the years. These approaches reveal a high level of polymorphisms and assisted in separation of landraces from commercial varieties and the presence of multiple genetic lineages. Importantly, marker-assisted characterization of genotypes would assist breeders in choosing genetically distant and stress-resilient parents for hybridization to improve yield and adaptability. Despite their utility in preliminary diversity assessment, dominant markers lack reproducibility and resolution, limiting their application in high-precision genetic mapping and modern breeding strategies; however, they continue to be used due to their low cost, simplicity, and independence from prior genomic information, particularly in resource-limited settings.

Co-dominant markers

To overcome these limitations, more reliable co-dominant markers such as SSR, SCARs, and SNP markers (Table 1) have been developed and applied in cluster bean [25-28,33].

SCAR Markers

SCAR systems have also been employed for cluster bean germplasm characterization (Table 1). For example, Sharma et al. [52] developed a SCAR marker system from polymorphic RAPD and ISSR-derived amplified bands to genotype 35 cluster bean accessions [52]. They selected RP-3 (1,000 bp), RP-19 (1,250 bp, 1,100 bp), and RP-9 (650 bp) from polymorphic RAPD markers and one ISSR amplicon of 550 bp from IS-8 marker in the RGC-1031 genotype. Among the 20 SCAR markers developed in the study, SCAR-20 was specific to genotype RGC 1031 (had tolerance to fungal pathogen *Macrophomina phaseolina*), and SCAR-8 amplified only the genotypes from Rajasthan (RGC-197, RGC-986, RGC-936, RGC-1002, RGC-1003, RGC-1031, RGC-1017, RGC-1053, RGC-1066, RGM-112, RGM-111, RGM-115, RGC-1030, Bundel guar). Hence, SCAR markers enabled the geographical authentication of genotypes and direct selection of disease-resistant plant without field screening.

SSR Markers

Expressed sequence tag-based SSRs: SSRs were initially identified in cluster bean from the 16,476 expressed sequence tags (ESTs) in the National Centre for Biotechnology Information (NCBI) database (Table 2) [61]. Thereafter, these ESTs have been widely used to identify and develop SSRs in cluster bean. Kuravadi et al. [25] designed 362 primer pairs from these ESTs and synthesized 224 markers for polymorphism analysis in five accessions of three *Cyamopsis* species. Out of 224 primer pairs, 184, 121, and 187 resulted in reproducible bands in *C. serrata*, *C. senegalensis*, and *C. tetragonoloba*, respectively. The study provided 187 SSR markers for the guar researchers. Similarly, Kumar et al. [54] generated 100 EST-derived novel primers pairs, of which 50 markers associated with gum biosynthesis genes were used for diversity analysis in 32 genotypes. The majority of genotypes clustered into three groups irrespective of geographical origin, indicating low genetic diversity. In addition to the EST data sets, expressed sequences from the RNA-Seq experiments were also used to identify SSR markers. For instance, Rawal et al. [18] identified 8,687 potential SSRs from tissue-specific transcriptome of *C. tetragonoloba* cv RGC-936. EST-derived SSRs have long served as a reliable and widely used marker resource in cluster bean research.

How to cite this article:

Genome-wide SSRs: Transcriptomic and genome sequencing approaches have led to the identification of thousands of SSRs. RNA-Seq analysis of two guar varieties, RGC-1066 and M-83, also yielded 5,773 potential SSRs [32]. A PCR validation using 21 SSRs resulted in 13 SSRs that showed amplification in both varieties [32]. In another study, RNA-Seq analysis of the roots of guar varieties, RGC-1066 and M-83, was carried out, and 18,792 SSRs were determined [62]. Comparative transcriptome data of cluster bean genotype BWP 5595 under salinity, drought, and heat stress was used to mine SSRs using microsatellite identification tool (MISA), and 27,066 SSRs were found with an average frequency of 1 SSR every 9.8 kb in the assembled transcripts [63]. Transcriptomic studies were conducted on drought-tolerant (RGC-1002) and susceptible (RGC-1066) cluster bean varieties, and the RNA-Seq data was assessed for SSR mining. A total of 22,212 and 14,650 SSRs were identified in the tolerant and susceptible varieties, respectively [64]. In a separate study, de novo transcriptome analysis of *C. tetragonoloba* cv RGC-1025 yielded 21,494 SSRs [65]. These studies highlighted the use of expressed sequences or transcriptome datasets to identify SSR markers. Additionally, unassembled MiSeq whole genome dataset was used to identify microsatellite repeat sequences from cluster bean variety GG-4 to design 15,399 SSR marker primers. A subset of 21 SSRs was used to validate the markers across 50 genotypes. A diversity analysis using these markers suggested that a low genetic variability is present in the guar gene pool [59]. Similarly, the whole genome sequence of *C. tetragonoloba* cv RGC-936 was used to identify 1,859 genomic SSRs from 1,091 scaffolds. Eighty-nine of the identified markers were validated using 54 cultivated guar accessions and two wild relatives, *C. serrata* and *C. senegalensis*. Approximately 92% of the tested SSRs were polymorphic in the 56 cultivars. The cultivated accessions of *C. tetragonoloba* formed four major clusters, while two wild accessions formed a separate cluster [28]. Later on, when the whole genome sequence (*C. tetragonoloba* cv RGC-936) data was published in 2023, it showed the presence of 238,176 SSRs in the cluster bean genome [31]. Overall, large number of genomic markers are valuable for identifying marker-trait associations. Priti et al. [57] had developed the first SSR-based linkage map of cluster bean using inter-specific populations and identified the quantitative trait loci (QTL) for important agronomic traits and disease resistance [57]. These markers provide opportunities to implement marker-assisted breeding strategies for development of superior cluster bean varieties. Recently, 20 SSR makers were used to assess genetic diversity in 21 cluster bean genotypes. The 19 SSRs that showed amplification generated 50 alleles, with number of alleles per locus ranging from 1 to 4. The study depicted a low genetic diversity with a mean polymorphism information content (PIC) value of 0.24. Clustering analysis generated three clusters, viz. A, B, and C. Most of the genotypes (16) clustered in group B, while only two and three genotypes clustered in clusters A and D, respectively. Genotypes DRLG-18 and DRLG-29 exhibited a maximum genetic dissimilarity of 0.4891 [58].

How to cite this article:

. P, Jangra S, Chaudhary V, et al. (May 20, 2026) Biotechnological Interventions in Unravelling the Genetic Treasure of an Orphan Crop, Cluster Bean (*Cyamopsis tetragonoloba* L.). Curues J. Agri. Food Sci 2 : es44497-025-00069-2. DOI <https://doi.org/10.7759/s44497-025-00069-2>

Genotypes/Variety/Database	No. of SSR markers developed	Technology used	Reference
16,476 ESTs	362	MicroSatellite (MISA)	[25]
16,476 ESTs	100	MISA	[54]
RGC-936 (Unigenes)	8,687	MISA	[18]
RGC-1066 and M-83 (Unigenes)	5,773	MISA	[32]
RGC-1066 and M-83 (Unigenes)	18,792	MISA	[62]
RGC-936 (whole genome sequence)	1,859	MISA	[28]
BWP 5595 (transcriptome)	27,066	MISA	[63]
GG-4 (genome sequence)	15,399	PrimerPro v1.0	[59]
RGC-1025 (transcriptome)	21,494	MISA	[65]
RGC-1066 (transcriptome)	14,650	MISA	[64]
RGC-1002 (transcriptome)	22,212	MISA	[64]
RGC-936 (genome)	238,176	MISA	[31]

TABLE 2: SSR markers developed for cluster bean.

Despite the large number of SSR markers identified, their application has largely remained confined to diversity analysis and low-resolution mapping. Integration of these markers into high-density linkage mapping and genomics-assisted breeding remains limited.

SNP Markers

SNPs are a dynamic class of molecular markers, as the polymorphism generated by transitions and transversions is tightly linked to phenotypic variations. SNPs are widely distributed in the genome over intergenic, coding, and non-coding regions. Along with SNPs, Indels are also an important class of markers that are responsible for structural variations in the genome and are extensively distributed over the genomes of crops like tomato, chickpea, rice, and *Arabidopsis*. To mine SNPs from the genome, various methods have been used, such as whole genome sequencing, comparative genomics, RNA-Seq, comparative transcriptomics, etc. Recently, mRNA and genome sequencing have also been used to identify SNPs and InDels in the cluster bean genome. For example, a comparative transcriptomic analysis of RGC1066 and M-83 varieties of *C. tetragonoloba* resulted in a total of 8,416 SNP markers with read depth values of more than 5 [32]. They chose varieties with contrasting endosperm gum content, which may help discover the SNPs associated with trait gum synthesis. Statistical analysis reflected that 65.25% of SNPs are due to transitions, and the rest are due to transversions in M-83. Likewise, in RGC-1066, 61.36% transitions and 38.64% transversions were observed to be responsible for the

How to cite this article:

. P, Jangra S, Chaudhary V, et al. (May 20, 2026) Biotechnological Interventions in Unravelling the Genetic Treasure of an Orphan Crop, Cluster Bean (*Cyamopsis tetragonoloba* L.). Curues J. Agri. Food Sci 2 : es44497-025-00069-2. DOI <https://doi.org/10.7759/s44497-025-00069-2>

generation of SNPs. Both varieties demonstrate a similar pattern of transition and transversions. In another study by Thakur and Randhawa [62], a comparison of mRNA sequences from the roots of RGC-1066 and M-83 genotypes revealed 5,999 SNPs and 249 InDels with read depth value >10 [62]. The study revealed that RGC-1066 and M-83 cluster bean genotypes comprise 2,479 and 2,870 SNPs, respectively. SNPs were found to be involved in root development, phytophthora resistance, galactomannan synthesis, and heat and cold stress (Table 3; Table 4). Among total InDels, insertions were more than deletions, found to be located on 153 and 87 unigenes, respectively. Longest insertion (17 bp) was found in gene involved in N-acetyl transferase activity, while the longest deletions (18 bp) were found in a gene involved in tRNA methyltransferase activity. Almost all of the markers were characterized with respect to location and functions. Sahu et al. [33] identified SNPs and InDels by assessing RNA-Seq data of three *C. tetragonoloba* cultivars, viz. RGC-936, RGC-1066, and M-18. Besides identification of markers, they also documented the presence of common and cultivar-specific SNPs and Indels. Additionally, an online database 'cbSIR' was developed based on cultivar-specific markers (http://webapp.cabgrid.res.in/clb_ce/index.php). Functional annotation of these SNPs/Indels reflected their localization in genes that control gum production, auxin transport, and disease resistance in cluster bean. Similarly, restriction site-associated DNA sequencing was used to identify SNP and InDel markers from 45 guar accessions from different geographical origins [60]. This analysis identified 4,907 SNPs and 327 InDels. All the information associated with identified SNPs and INDELS was stored and organized in an online repository named 'Cluster bean SNPs and INDELS Repository-cbSIR'. SNPs and InDels provide high-resolution, functionally annotated markers associated with important traits such as gum biosynthesis, stress tolerance, and root development, making them highly valuable for genetic mapping and breeding applications in cluster bean. Despite significant advances in SNP discovery, their application in cluster bean breeding remains limited due to insufficient marker density, small population sizes, and lack of standardized genomic resources.

How to cite this article:

. P, Jangra S, Chaudhary V, et al. (May 20, 2026) Biotechnological Interventions in Unravelling the Genetic Treasure of an Orphan Crop, Cluster Bean (*Cyamopsis tetragonoloba* L.). Curues J. Agri. Food Sci 2 : es44497-025-00069-2. DOI <https://doi.org/10.7759/s44497-025-00069-2>

Discovery method	Variety	Application	Tissue type	Validation	No. of SNPs identified	Functional annotation/ trait associated	Reference
Comparative transcriptomics	RGC1066 and M-83	-	leaf	-	3,594 SNP markers	-	[32]
Comparative transcriptomics	RGC1066 and M-83	-	root	5 randomly selected SNPs validated	5,999 SNPs	Galactomannan synthesis, defence response, and heat and cold stress response, negative regulation of p53	[62]
RNA-seq data from SRA	RGC-936, RGC-1066, and M-18	-	-	80% of the total markers	Total number of SNPs in M-83, RGC-1066 and RGC-936 are found to be 69,906, 70,886 and 46,699	Gum production, disease resistance, root development	[33]
Genotyping-by-sequencing restriction site-associated DNA sequencing	45 accessions of guar	MAS	-	6 loci	4,907 common SNPs	Percentage of pods matured to the harvest date under long light day conditions, transcription factor MYB20, F-box/LRR-repeat protein 3, ABC transporter G family member 39, glucuronosyl transferase,	[60]

How to cite this article:

. P, Jangra S, Chaudhary V, et al. (May 20, 2026) Biotechnological Interventions in Unravelling the Genetic Treasure of an Orphan Crop, Cluster Bean (*Cyamopsis tetragonoloba* L.). Curues J. Agri. Food Sci 2 : es44497-025-00069-2. DOI <https://doi.org/10.7759/s44497-025-00069-2>

						glycine cleavage system H protein 2, SECA2, protein translocase subunit	
GBS	RGC-936/CAZRI-15-3-8	Linkage map	leaf		6,113 SNP markers	-	[31]
GBS	225 guar genotypes	Genetic diversity and population structure analysis	leaf	-	7,000	-	[19]
GBS	225 guar genotypes	GWAS	leaf	-	19,007 filtered SNPs	Plant height, nodule number per plant, fresh nodule weight, dry nodule weight, fresh aboveground plant biomass, and dry aboveground plant biomass	[19]

TABLE 3: Studies on SNPs detection, validation, and application in cluster bean.

SNP, Single-Nucleotide Polymorphism; SRA, Sequence Read Archive; GBS, Genotype-by-Sequencing; GWAS, Genome-Wide Association Studies; MAS, Marker-Assisted Selection

How to cite this article:

. P, Jangra S, Chaudhary V, et al. (May 20, 2026) Biotechnological Interventions in Unravelling the Genetic Treasure of an Orphan Crop, Cluster Bean (*Cyamopsis tetragonoloba* L.). Curues J. Agri. Food Sci 2 : es44497-025-00069-2. DOI <https://doi.org/10.7759/s44497-025-00069-2>

Category	Marker positions/ID	Associated trait	Gene/transcript	Function	References
Genome-based SNPs (Chr)	Chr2:51078296, Chr2:51010513, and Chr5:55996909	Plant height	–	-	[19]
	Chr5:55996909	Plant height	Transcription nuclear factor Y (NF-Y) alpha-related	Growth regulation	[19]
	Chr3:13052718	Nodule number per plant	–	-	[19]
	Chr6:56449898	Nodule number per plant	Vacuolar iron transporter-like	Nodulation	[19]
	Chr3:39258868	Nodule diameter	–	-	[19]
	Chr5:35619658	Fresh Nodule Weigh	Nodulin protein	Nodule development	[19]
	Chr2:51078296	Fresh Nodule Weigh	Calcium-binding protein	Signaling	[19]
	Chr3:7773837, Chr7:12878334, Chr1:48466801	Fresh Nodule Weigh	–	-	[19]
	Chr6:56449898	Dry nodule weight	Vacuolar iron transporter-like	Nodulation	[19]
	Chr2:50961410, Chr3:29368549, Chr3:30748062, Chr3:31743839, Chr4:20715064, Chr4:4985600, Chr6:13748571	Dry nodule weight	–	-	[19]
	Chr2:51078296	Above-ground biomass	Calcium-binding protein	Growth regulation	[19]
	Chr2:51010513	Above-ground biomass	Purple acid phosphatase	Nutrient metabolism	[19]
	Chr2:50910112,	Above-ground biomass	–	-	[19]

How to cite this article:

. P, Jangra S, Chaudhary V, et al. (May 20, 2026) Biotechnological Interventions in Unravelling the Genetic Treasure of an Orphan Crop, Cluster Bean (*Cyamopsis tetragonoloba* L.). Curues J. Agri. Food Sci 2 : es44497-025-00069-2. DOI <https://doi.org/10.7759/s44497-025-00069-2>

	Chr2:51010513	Dry above-ground biomass	Purple acid phosphatase	Nutrient metabolism	[19]
Transcriptome-based (Contig)	Contig59:14389378	Percentage of pods matured to harvest date	Transcription factor MYB20	-	[60]
	Contig37:351180	Percentage of pods matured to harvest date	SECA2, Protein translocase subunit	Protein transport	[60]
	Contig229:330388	Percentage of pods matured to harvest date	Glycine cleavage system H protein 2	Metabolism	[60]
	Contig88:4931265	Percentage of pods matured to harvest date	Putative glucuronosyltransferase	Cell wall metabolism	[60]
	Contig182:841660	Percentage of pods matured to harvest date	ATP-binding cassette (ABC) transporter G family member 39	Transport	[60]
	Contig188:3026232	Percentage of pods matured to harvest date	F-box/Leucine-rich repeat (LRR)-repeat protein 3	Protein turnover	[60]
Candidate gene (RNA-Seq)	OT2789-OT2794, OT3537	-	Cellulose synthase A	Mannan biosynthesis/Transportation of mannose, nucleotide-sugar transport	[62]
	OT4846, OT1382	-	Cellulose synthase-like proteins	Transportation of mannose, nucleotide-sugar transport	[62]
	OT3553-OT3555	-	Receptor-like protein kinase HSL1-like isoform X1	Regulation of root development	[62]
	OT3861-OT3863	-	Uncharacterized protein LOC100780184	Root cell wall biosynthesis	[62]

How to cite this article:

. P, Jangra S, Chaudhary V, et al. (May 20, 2026) Biotechnological Interventions in Unravelling the Genetic Treasure of an Orphan Crop, Cluster Bean (*Cyamopsis tetragonoloba* L.). Curues J. Agri. Food Sci 2 : es44497-025-00069-2. DOI <https://doi.org/10.7759/s44497-025-00069-2>

	OT4656	-	Cellulose synthase-like protein B3-like	Root hair morphogenesis	[62]
	OT769, OT1113-OT1116, OT1881, OT1916	-	Nucleotide-binding site (NBS)-LRR type, Disease resistance protein, Resistance gene analog 2 (RGA2), Nucleotide-binding adaptor shared by APAF-1, R proteins, and CED-4 (NB-ARC) domain disease resistance protein	Disease resistance	[62]
	OT1431-OT1437	-	Disease resistance RPP13-like protein 1	Plant hypersensitive response, disease resistance	[62]
	OT2923, OT2924	-	S-norococlaurine synthase	Pathogen-related (PR) 10 protein, defense response	[62]
	OT3720	-	RING finger and CHY zinc finger domain-containing protein	Negative regulation of p53	[62]
	OT67	-	KK1_005680	Response to freezing	[62]
	OT458, OT543, OT3934, OT3935, OT1691, OT1738	-	DNAJ protein homolog, Heat shock factor protein HSF24, Heat stress transcription factor A-2-like, A-5, and A-4a like	Response to heat stress	[62]
	OT697-OT698, OT1715, OT4075, OT4076, OT4342	-	Callose synthase	Control delivery of UDP-Glc to the synthase	[62]
	OT2742-OT2747	-	Cellulose synthase A catalytic subunit 1 [UDP-forming]	Mannan biosynthesis	[62]

How to cite this article:

. P, Jangra S, Chaudhary V, et al. (May 20, 2026) Biotechnological Interventions in Unravelling the Genetic Treasure of an Orphan Crop, Cluster Bean (*Cyamopsis tetragonoloba* L.). Curues J. Agri. Food Sci 2 : es44497-025-00069-2. DOI <https://doi.org/10.7759/s44497-025-00069-2>

	OT35553-OT3555	-	Receptor-like protein kinase HSL1-like isoform X1	Regulation of root development	[62]
	OT3861-OT3863	-	Uncharacterized protein LOC100780184	Root cell wall biosynthesis	[62]
	OT4656	-	Cellulose synthase-like protein B3-like	Root hair morphogenesis	[62]

TABLE 4: Genome-wide SNPs, transcriptome-derived loci, and candidate gene-associated SNPs linked to key traits in cluster bean.

SNPs, Single-Nucleotide Polymorphisms

Molecular markers (SSRs and SNPs) enabled analysis of complex trait loci

Many agronomic traits are quantitative in nature. Improving crops through conventional breeding usually takes time and is a labor-intensive practice. Molecular markers linked to desired traits can aid in marker-assisted selection to efficiently screen large populations in breeding trials. Hence, marker-assisted selection can significantly improve the selection efficiency in the breeding programs by limiting the efforts and time consumption for phenotypic data assessments.

Biparental Mapping

Fewer studies have mapped and identified the biotic stress resistance genomic region in cluster bean. For instance, Gaikwad et al. [31] generated a high-density linkage map of cluster bean using SNP markers. They utilized an F2 population derived from RGC-936 and CAZRI-15-3-8 cluster bean genotypes and employed genotyping-by-sequencing to obtain 6,113 markers for linkage map construction. The final map consisted of seven linkage groups at a logarithm of odds score of 6.0, but they did not report any QTL detection in their study.

Priti et al. [57] constructed a linkage map spanning 190 cM of the cluster bean genome using microsatellite markers in an interspecific cross between a BLB-resistant wild species (*C. serrata*) and a moderately susceptible cultivar (HG563). A total of 177 SSR markers were used, derived from cluster bean and related legumes. These included 11 EST-SSRs associated with disease resistance, 36 linked to gum content, and 45 developed from publicly available EST datasets, while the remaining markers were sourced from other legumes such as adzuki bean, common bean, pigeon pea, and mung bean. Out of 177 SSRs, 38 were found polymorphic and were utilized for linkage map and QTL analysis. A total of nine EST SSRs from cluster bean were found to be linked on single linkage group spanning 190 cM. Furthermore, the QTL mapping led to the identification of four minor QTLs for plant height, hundred seed weight, disease resistance, and gum content with logarithm of odds values of 3.3, 12.5, 4.7, and 15.4, respectively. In addition, the above study identified three lines, viz. 2-8, 5-11, and 6-9, that were selected on the basis of agronomical trait data and BLB disease resistance under field conditions. Similarly, Bajaj and Pahuja [50] identified QTL associated with BLB in cluster bean using RAPD markers [50]. A F2-3 population derived from HG75×PNB was used for QTL analysis. They identified one major QTL (BKSLVI), with 11% of variation in disease resistance. While QTLs BKSLVD and BKSLVF were detected with 4% of total variation for disease resistance. RAPD markers L19, D1, and AB7 showed strong linkage with resistance traits, forming a linkage map spanning 43.9 cM. The identified QTL offers a potential target for future cluster bean improvement programs.

Genome-Wide Association Studies (GWAS)

How to cite this article:

. P, Jangra S, Chaudhary V, et al. (May 20, 2026) Biotechnological Interventions in Unravelling the Genetic Treasure of an Orphan Crop, Cluster Bean (*Cyamopsis tetragonoloba* L.). Curues J. Agri. Food Sci 2 : es44497-025-00069-2. DOI <https://doi.org/10.7759/s44497-025-00069-2>

GWAS provides an alternative to general bi-parental or multi-parental QTL mapping by capturing variation in natural crop populations. This approach has successfully identified molecular markers and genes for many complex traits in different crop species. Until now, GWAS studies were limited in cluster bean due to several factors, including (1) the absence of a fully sequenced and well-annotated genome and (2) lack of a substantial number of genetic markers dispersed across the entire genome to enable a high-resolution analysis of marker-trait associations.

Until now, only two GWAS studies have been reported in cluster bean. Aditi [55] performed GWAS in 158 cluster bean genotypes that were cultivated at two different locations for morphological, agronomic, and yield-related traits. A total of 218 EST-SSR markers were used, primarily developed from the EST database reported by Naoumkina et al. [61]. Among these, 30 EST-SSRs were derived from pathogen stress-related ESTs, and 20 degenerate primers were designed from conserved motifs of NBS-LRR resistance genes. Additionally, 103 EST-SSR markers specific to cluster bean were included, along with a subset of markers sourced from other legumes, out of which, total 85 SSRs were used for diversity, population structure, and marker-trait association analysis. Analysis of population structure identified two main clusters with K=2 and eight possible subgroups. Further GWAS analysis revealed a total of 22 marker-trait associations using TASSEL (2.1) at $P < 0.05$. Three markers (CBN22, CTF229, and GDR8) were found to be associated with seed yield.

In another study, GWAS was conducted using 45 guar accessions and SNP markers generated from the GBS RADseq platform [60]. They identified six markers linked to the percentage of pods matured to harvest date, and one marker was found to be homologous to the ABC transporter gene in *Arabidopsis*. This gene controls the metabolism of D-myo-inositol phosphates. An earlier report has stated that the genes involved in the metabolism of D-myo-inositol phosphate also impact early flowering in cluster bean and are observed in higher concentration in tissues of the third true leaf [66]. With new advances in cluster bean genomics and molecular biology, further studies are expected to expand GWAS applications to understand the genetics of complex traits in this crop. It has been observed that nodule number is unaffected in drought stress, though nodule fresh weight and nitrogenase activity decreased in response to drought stress. Nodule biomass is slightly controlled by both environmental and genetic factors [67]. Thus, identifying molecular markers associated with desired nodule traits will enhance the comprehension of the genetic architecture of nodulation and nitrogen fixation in the guar crop. A study has been conducted for trait associations in guar to identify SNPs associated with nodules in guar [19]. The SNPs have been identified with plant height, nodule number per plant, fresh and dry nodule weight, and fresh and dry above-ground biomass, which could be a beneficial tool in the selection of better nodule traits in guar. A GWAS conducted on 225 guar genotypes using 19,007 SNP markers identified loci associated with nodulation and related traits [19]. Using multiple statistical models implemented in TASSEL, significant SNP associations were detected for plant height, nodule number, fresh and dry nodule weight, and aboveground biomass. Notably, a higher number of associations were observed for nodule weight traits, indicating their strong genetic basis. These findings provide initial evidence for the genetic control of nodulation-related traits in cluster bean and highlight the potential of SNP markers for improving nitrogen fixation efficiency.

SNPs and InDels represent a highly informative and abundant class of molecular markers in cluster bean, as they are widely distributed across coding and non-coding regions and are closely associated with functional trait variation. Advances in high-throughput approaches such as RNA-Seq, comparative transcriptome, and genome sequencing have enabled the identification of thousands to millions of SNPs and InDels, greatly enhancing marker density. These markers have been successfully linked to key agronomic and stress-related traits, including gum synthesis, root development, disease resistance, and abiotic stress tolerance. For example, comparative transcriptome studies identified thousands of SNPs differentiating genotypes with contrasting traits (e.g., gum content), while GBS and RAD-Seq approaches enabled the genetic diversity analysis, linkage mapping, and GWAS. Moreover, the development of databases such as cbSIR and the identification of trait-associated SNP loci provide valuable resources for marker-assisted selection and precise breeding.

Despite recent progress, molecular marker applications in cluster bean remain underdeveloped. Although SSR- and SNP-based markers have enabled the identification of QTLs and marker-trait associations, most studies rely on low-density markers and limited populations. Furthermore, many identified QTLs lack validation across environments and genetic

How to cite this article:

. P, Jangra S, Chaudhary V, et al. (May 20, 2026) Biotechnological Interventions in Unravelling the Genetic Treasure of an Orphan Crop, Cluster Bean (*Cyamopsis tetragonoloba* L.). *Curues J. Agri. Food Sci* 2 : es44497-025-00069-2. DOI <https://doi.org/10.7759/s44497-025-00069-2>

backgrounds, limiting their direct application in marker-assisted selection. The integration of high-density SNP datasets, well-structured mapping populations, and genome-wide analyses will be essential to bridge the gap between marker discovery and practical breeding.

Omics tools to facilitate cluster bean improvement

Unlike other legume crops, cluster bean is lagging in marker and gene discovery efforts and its potential use in breeding. This is largely due to a lack of enough genomic information since very few studies have employed omics technologies for the identification and understanding of the genetic elements for desirable traits in this crop. However, the use of omics tools has increased in cluster bean, and efforts towards genome sequencing of cluster bean were made to uncover the full potential of the crop (Figure 2) [30,31]. However, the translation of these omics-derived insights into field-level breeding applications remains limited.

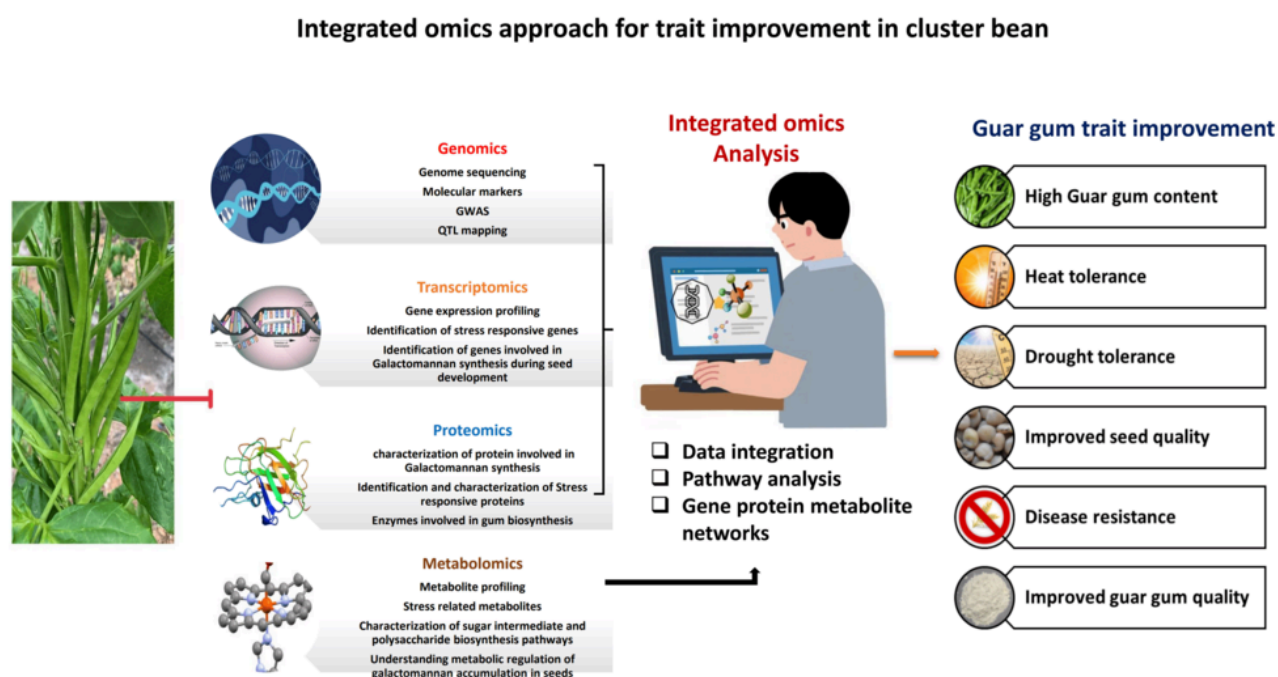


FIGURE 2: Integrated omics framework for trait improvement in cluster bean, linking multi-layer molecular data to key agronomic traits through data integration and pathway analysis.

Cluster bean organelle and nuclear genome assemblies

A plant cell harbors three different genomes in mitochondria, chloroplasts (Cp), and the nucleus. Among these, the chloroplast genome is more conserved and widely employed for phylogenetic studies to explore the evolutionary origin and relative age of different species [68]. Cp genome is maternally inherited and is less prone to recombination. Therefore, Cp genome is a potent indicator of organism's age in contrast to nuclear genomes. Cp genome of cluster bean is quadripartite in structure with two copies of inverted repeats (IR) spaced with long single copy (LSC) and small single copy (SSC) region [69]. IR is mainly responsible for reduction or expansion of organelle genome size, thus influencing the Cp genome size. Cp genome of guar (RGC936) was assembled by Kaila et al. [29], with an estimated genome size of 152,530 bp. It had 83,025 bps of LSC, 17,879 bps of SSC, and 25,790 bps of both IR. Cluster bean Cp genome consisted of 114 unique genes comprising 4 rRNA genes, 30 tRNA genes, 2 pseudogenes, and 78 protein-coding genes. An expanded IR region was present in cluster bean Cp genome that contains whole 19 ribosomal proteins (rps) genes as compared to other legume plastid genomes. Guar Cp genome is reported to have undergone expansion as the whole rps19 gene is included in IR when compared with other legumes.

How to cite this article:

. P, Jangra S, Chaudhary V, et al. (May 20, 2026) Biotechnological Interventions in Unravelling the Genetic Treasure of an Orphan Crop, Cluster Bean (*Cyamopsis tetragonoloba* L.). Curues J. Agri. Food Sci 2 : es44497-025-00069-2. DOI <https://doi.org/10.7759/s44497-025-00069-2>

Cluster bean has a diploid genome, which is distributed across 14 chromosomes ($2n=14$) [70–72]. In 2019, Tyagi et al. [73] estimated the DNA content of three *Cyamopsis* species (*C. tetragonoloba*, $1.18a \pm 0.02\text{pg}$; *C. serrata* $2.00a \pm 0.02\text{pg}$; *C. senegalensis* $1.92a \pm 0.03\text{pg}$) with flow cytometry, and by taking soybean as a standard. The average nuclear genome size was estimated as *C. tetragonoloba* 580.9 Mbp (1C), *C. serrata* 979.6 Mbp (1C), and *C. senegalensis* 943.4 Mbp (1C) [73]. These results indicated that wild relatives of cluster bean had almost two-fold DNA content than the cultivated species *C. tetragonoloba*.

Until 2019, no reference genome was available for cluster bean. The very first reference genome for cluster bean was reported for ‘Vavilovskij 130’ accession [30], which is highly recommended in southern regions of Russia for propagation. This particular genome was sequenced using Illumina short reads and Oxford nanopore long reads methods, and the final assembly was >1 Gb, representing approximately 50% of the genome. Later, Gaikwad et al. [31] reported for the first time chromosome-scale assembly of a popular high gum content cultivar of cluster bean, i.e., RGC-936, by employing third-generation sequencing technologies like Illumina and Oxford Nanopore. They generated 201.8 Gb of raw sequencing data with 366.73X genomic coverage of the cluster bean genome. This data was further improved with a total of 1,580 scaffolds spanning 550.16 Mb. A total of 99.8% (549.23 Mb) of the assembled genome was assigned to seven linkage groups with a size range of 61.32 to 93.95 Mb and an N50 value of 78.27 Mb. Li et al. [74] further enriched cluster bean genome resources by sequencing a short-day variety of *C. tetragonoloba* and generated raw sequence data of about 57.21 Gb. Finally, the assembled genome size was 482.45 Mb with an N50 value of 62.64 Mb. They were able to assemble 99.07% (477.96 Mb) of the sequencing data into seven pseudochromosomes. A total of 31,251 genes out of 31,867 were efficiently annotated, and as a result, 559 gene families expanded, and 309 gene families contracted in *C. tetragonoloba*. These genomic resources provide a foundation for identifying genes and markers associated with key agronomic and industrial traits, including gum biosynthesis, stress tolerance, and adaptation. Their integration with transcriptomic and metabolomic datasets will be critical for advancing genomics-assisted breeding in cluster bean.

Transcriptomics

Comparative transcriptomics has also been carried out in cluster bean to understand the physiological and genetic mechanisms of stress tolerance [63,65,75,76]. These studies compared the gene expression patterns between stressed and non-stressed plants to identify candidate genes and their regulatory pathways. They studied their role in mitigating biotic and abiotic stresses in cluster bean. For example, a transcriptome analysis of *C. tetragonoloba* accession ‘BWP 5595’ leaves under stressed (water, temperature, salt stress) and non-stressed conditions generated 193.5 M high-quality reads. A de novo transcriptome assembly generated 161,058 transcripts and identified 61,508 putative genes. A functional annotation analysis indicated that these transcripts were associated with 985 enzymes and 156 different pathways [63]. The same accession was also used to identify genes and pathways involved in heat stress tolerance in cluster bean. A comparative differential gene expression analysis between control and stressed plants identified 1,551 upregulated and 1,466 down-regulated genes after heat stress. A functional analysis linked the differentially expressed genes to 158 enzymatic proteins and 102 molecular pathways, some of which include heat shock protein (hsp) families like Hsp20, Hsp70, Hsp90, and a heat shock transcription factor family (IPR027725) [77]. A gene expression comparison was also conducted using RNA-seq to identify the drought-responsive genes in the same accession of *C. tetragonoloba*. A total of 499 and 191 genes were upregulated and downregulated, respectively, after the drought stress treatment. From these differently expressed genes, 38 genes (32 up-regulated and 6 down-regulated) were recognized as novel guar genes. In addition, up-regulation of two IPS sites, 306 domains, 12 repeats, and 137 protein families was observed. The proton-dependent oligopeptide transport and transferase families, major intrinsic protein, aspartic peptidase A1 family, calcium/calmodulin-dependent/calcium-dependent protein kinase, aquaporin transporter, and UDP-glucuronosyl/UDP-glucosyltransferase were highly upregulated. KEGG analysis unveiled that the up-regulated unigenes represent 88 enzymes. Furthermore, the ERF, MYB, and MYB-related transcription factor families were also up-regulated under drought stress conditions [78]. The drought stress transcriptomics was also conducted in a different cluster bean cultivar, ‘RGC-1025’. A de novo assembly of RNA-Seq data resulted in a total of 66,838 transcripts involving 203 pathways. Differential gene expression (DGEs) analysis of the transcripts showed the upregulation of previously known drought-responsive genes such as low temperature-induced 65 kDa protein (LDI65), alpha dioxygenase 2, putative vacuolar amino acid

How to cite this article:

. P, Jangra S, Chaudhary V, et al. (May 20, 2026) Biotechnological Interventions in Unravelling the Genetic Treasure of an Orphan Crop, Cluster Bean (*Cyamopsis tetragonoloba* L.). *Curues J. Agri. Food Sci* 2 : es44497-025-00069-2. DOI <https://doi.org/10.7759/s44497-025-00069-2>

transporter, and late embryogenesis abundant protein 3 (LEA 3). Up-regulation of stress-responsive MYB, WRKY, NAC, and GRAS transcription factor families was also observed. The study also showed that the wax deposition on guar leaves can provide tolerance to drought stress [65], and wax biosynthesis genes were present in the DEGs identified from this study.

To get deeper insights into the drought tolerance in cluster bean, a comparative transcriptome analysis was conducted between drought-tolerant 'RGC-1002' and susceptible 'RGC-1066' genotypes after treating the plants with drought stress for 30 days. A DEG analysis showed an up-regulation of 5,206 and 6,625 transcripts in tolerant and susceptible genotypes, respectively. Genes like transketolase family protein, phosphoenolpyruvate carboxylase 3, temperature-induced lipocalin, and cytochrome oxidase were significantly up-regulated in the tolerant genotype. A total of 2,793 and 5,365 transcripts were downregulated in tolerant and susceptible genotypes, respectively. A KEGG analysis further revealed that starch and sucrose metabolism-related genes may be associated with drought tolerance in cluster bean [64]. All these studies led to the identification of several putative genes and transcription factors that can be utilized for improving drought resilience in cluster bean.

Salt stress is another factor that limits cluster bean production and yield [79]. To decipher the molecular mechanism of salt stress tolerance, transcriptomes of tolerant 'Matador' and susceptible 'PI 340261' cluster bean genotypes were compared using RNA-Seq approach. Sequencing of leaf and root tissue of plants under stressed and controlled conditions yielded 527 million clean reads from which 296,114 unigenes were assembled. DEG analysis showed that the genes involved in transporters, chromatin remodeling, stress-signaling, translational machinery, and microRNA biogenesis pathways play a key role in guar salt stress tolerance. Additionally, P-ATPase, ABC, GPH, and MFS transporter family genes were differentially expressed under salt stress conditions. The analysis further identified differential expression of SNF2, C2H2, MYB, bHLH, and C3H transcription factors/regulators under salt stress conditions [80].

The cluster bean transcriptomic studies consistently reveal coordinated regulation of stress-responsive genes, transport systems, and transcription factors, and showed that transcriptomics is a valuable tool to study stress responses. These datasets provide preliminary information to characterize the role of selected DEGs in conferring stress tolerance in cluster bean. However, most findings remain at the discovery stage, and functional validation of candidate genes is needed.

Metabolomics

Metabolomics delves into the comprehensive analysis of small molecules or metabolites in plant tissues. Plants produce unique metabolites during development and under biotic and abiotic stress conditions. Hence, metabolite profiling can provide valuable insights into plant development and stress resilience mechanisms [41] that can be leveraged for marker-assisted breeding strategies. Two complementary approaches are commonly used: untargeted metabolomics, which captures a broad spectrum of metabolites for pathway discovery but is often limited by challenges in metabolite identification, quantification accuracy, and reproducibility, and targeted metabolomics, which enables precise quantification of predefined metabolites with high sensitivity and reproducibility but is constrained by prior knowledge. While untargeted approaches are valuable for hypothesis generation, their direct application in breeding is limited unless followed by targeted validation. Therefore, integrating untargeted discovery with targeted validation is essential for identifying robust biomarkers associated with key traits such as galactomannan biosynthesis and stress tolerance in cluster bean.

Despite its potential, metabolomics research in guar remains limited and largely exploratory, with studies primarily focusing on gum biosynthesis, flowering behavior, and drought stress response. Comparative metabolome of immature seeds belonging to gum-type 'HGS 563' and vegetable-type 'Pusa Navbahar' (PNB) genotypes identified key metabolites involved in gum synthesis in guar [81]. GC-MS analysis of seeds reflected a higher accumulation of mannose and galactose in 'HG563' than 'PNB', which serves as the building block for galactomannan synthesis. Other sugars like fructose, raffinose, glucose, and xylose were also accumulated in higher concentrations in 'HG563' than 'PNB', but the accumulation of arabinose and rhamnose was the same in both genotypes.

Metabolomics has also been applied to dissect flowering time variation in 96 cluster bean genotypes grown under long daylight conditions [66]. Flowering time is an important agronomic trait that determines crop yield. Flowering time is highly influenced by environmental conditions, and identification of metabolites for early flowering can assist in

How to cite this article:

. P, Jangra S, Chaudhary V, et al. (May 20, 2026) Biotechnological Interventions in Unravelling the Genetic Treasure of an Orphan Crop, Cluster Bean (*Cyamopsis tetragonoloba* L.). *Curues J. Agri. Food Sci* 2 : es44497-025-00069-2. DOI <https://doi.org/10.7759/s44497-025-00069-2>

generating early-maturing guar genotypes. Early-maturing varieties can enable early harvest to avoid detrimental environmental conditions. Metabolomic profiling using gas chromatography-mass spectrometry revealed high concentrations of 65 metabolites in early than the late flowering genotypes in cluster bean [66]. These key metabolites represented tetronic, liquiritigenin, cinnamic acid molecules, chiro-inositol, and myoinositol (inositol isomers), which play an important role in early stages of plant embryogenesis [82].

Cluster bean is a hardy plant, but still faces yield constraints due to drought stress. Thus, the identification of key metabolites and biomarkers can be beneficial in improving drought resistance of the guar cultivar. Sharma et al. [83] used the gas chromatography-mass spectrometry technique to analyse the metabolites in response to drought stress in HG-563, a drought-tolerant cultivar of guar. They analysed the metabolic profiling of drought-treated HG563 and observed 23 altered metabolites. Eight metabolites were identified as biomarkers, viz. valine, proline, D-pinitol, palmitic acid, dodecanoic acid, glucose, threonine, and glycerol monostearate. Additionally, four amino acids (alanine, serine, valine, and aspartic acid) were found to play a significant role in drought stress tolerance. In another study, changes in seed metabolite content caused by drought stress in three guar genotypes (tolerant, neutral, and sensitive to drought) were studied to gain insight into the tolerance mechanism [8]. Drought stress led to increased monosaccharides and reduced glycosides across all genotypes; however, the tolerant genotype showed distinct accumulation of galactinol and higher levels of tocopherols, indicating enhanced osmoprotection and antioxidative capacity. In contrast, neutral and sensitive genotypes exhibited accumulation of glycosides, pentoses, phytosterols, and unsaturated C18 fatty acids. Overall, metabolites such as galactinol, phytol, and α -tocopherol consistently emerged as key contributors to drought tolerance.

Collectively, these insights provide a foundation for translating metabolic signatures into actionable targets for crop improvement by linking metabolite variation with underlying genetic mechanisms; however, their integration into breeding programs through validated metabolite markers and genotype-metabolite associations remains largely unexplored.

Integrated transcriptome-metabolome analysis

Photoperiod insensitivity is the desired trait to enhance the productivity of the crop. However, progress in breeding for this trait has been limited by insufficient functional markers and incomplete understanding of its genetic basis. Integration of multi-omics approaches, particularly transcriptomics and metabolomics, provides a powerful framework to dissect complex traits such as flowering time. Grigoreva et al. [76] made an effort to integrate transcriptomics and metabolomics to undermine the genetic architecture of variation in the flowering time of different cluster bean genotypes. They evaluated six delayed and nine early flowering varieties to find a connection between 1,067 differentially expressed transcripts and 63 metabolites using the Shiny GAM approach. A key biomarker of early flowering in cluster bean, myoinositol, was evaluated using the KEGG pathway available for *A. thaliana*. They observed that the phosphatidylinositol signaling pathway is initiated in plants that were ready for flowering, resulting in an exponential increase in myo-inositol in its free form as observed on the GC-MS chromatogram. The signaling pathway is performed by suppression of myoinositol phosphate kinase and overexpression of phosphatase.

In a separate study, metabolic profiling of three Indian genotypes contrasting for drought response, k-52938 (Tolerant), k-52924 (sensitive), and k-52891 (neutral), identified galactinol, phytol, and alpha-tocopherol as metabolites associated with drought tolerance in guar [8]. These compounds are linked to osmoprotection and oxidative stress mitigation, indicating their role in stress adaptation. Together, these studies demonstrate the potential of integrated transcriptome-metabolome approaches to identify candidate genes and metabolic biomarkers associated with key agronomic traits. Such insights can facilitate the development of functional markers for flowering time and stress tolerance, thereby supporting genomics-assisted breeding in cluster bean.

Implications of microRNAs (miRNA)

RNA is a major macromolecule that is responsible for translating genetic information from DNA into proteins. Apart from protein synthesis, regulating and expressing genes is a crucial function of RNA. These attributes of RNA can eventually determine the phenotypic expression of a trait. There are various types of RNA, each with its specific function in the cell. For example, small interfering RNAs (siRNAs), miRNAs, and long non-coding RNAs (lncRNAs) regulate gene expression

How to cite this article:

. P, Jangra S, Chaudhary V, et al. (May 20, 2026) Biotechnological Interventions in Unravelling the Genetic Treasure of an Orphan Crop, Cluster Bean (*Cyamopsis tetragonoloba* L.). Curues J. Agri. Food Sci 2 : es44497-025-00069-2. DOI <https://doi.org/10.7759/s44497-025-00069-2>

and maintain cell function. Although miRNAs are highly conserved among the plant and animal kingdoms, yet no sequence similarity has been observed among individual miRNAs across two distinct evolutionary lineages [84]. MiRNAs are subsequently involved in the regulation of genes in a wide range of organisms, including humans, insects, mammals, and plants. Understanding miRNAs and their target genes offers insights into biological events regulated by them.

MiRNAs are essential for the growth, development, and defensive responses of plants because they regulate various physiological processes [85]. Plant miRNAs are highly conserved and exhibit strong complementarity with the target sites. Extensive studies across species have demonstrated their roles in flowering, defense, and hormone signaling [86,87]. The miR156/miR172 pathways, for instance, are crucial for blooming time and pattern. Both long and short days cause blooming time to be delayed when miR156 overexpression is present. Through the repression of miR156 targets, floral homeotic gene APETALA2 (AP2)-like genes prevent Flowering Locus T (FT) from being expressed, thereby delaying floral transition. Other studies have shown that miR172 expression regulates AP2 genes by translational inhibition, thereby functioning as a flowering promoter [88,89]. Furthermore, the role of miR482/2118 in enhancing plant resistance against various diseases has been documented in many plant species [90]. Depending on the pathogen and species, the miR482/2118 has a positive or negative regulatory effect [91]. Numerous miRNAs function by interacting with hormones to control plant growth and development. miRNAs target several hormone-signaling components and manage hormonal responses by serving as intermediates in this signaling process. Alternatively, hormones can be used to regulate particular amounts of miRNA [92].

Beyond these general roles, recent studies on cluster bean have begun to explore the role of miRNAs in galactomannan biosynthesis. Tyagi et al. [35] performed RNA-Seq to understand the role of miRNA in galactomannan biosynthesis. Five different tissue types (root, shoot, stem, bud, leaf) from the genotype 'RGC-936' were selected for small RNA library preparation, and 187 known and 171 novel miRNAs exhibiting differential expression were identified. Out of the 15 miRNA families that were analysed in the seed tissue, miRNA159 was the most prevalent. It was followed by miR166, miR156, miR319, miR167, and miR168, which accounted for 71.3%, 10.9%, 2.8%, 0.4%, 0.3%, and 0.3%, respectively. In addition, three new miRNAs, Ct-miR3130, Ct-miR3135, and Ct-miR3157, were all found to be expressed in stem, and Ct-miR3135 was also found to be differentially expressed in seed. These miRNAs were validated as the targets of two novel unigenes, ManS (mannosyltransferase/mannan synthase) and UGE (UDP-D-glucose 4 epimerase), suggesting their potent role in galactomannan synthesis regulation.

Similarly, the transcriptome data of cluster bean variety 'M-83' was used to identify lncRNAs, miRNAs, and endogenous target mimics (eTMs) in cluster bean [34]. A total of 1101 lncRNAs and 22 miRNAs were identified. They also predicted the eTMs by finding the complementary base pairing between lncRNA and miRNAs. They identified eTMs like Ct_lnc_1644, Ct_lnc_5534, which acted as targets for miRNA-mir824, while Ct_lnc_1751, Ct_lnc_7639, and Ct_lnc_11423 were found to target miRNA mir168, mir7780p, and mi5640, respectively. It seems the identified eTMs are protecting mRNA for AGO1 [93], MADS-box transcription factors [94] and phosphoenol pyruvate carboxylase genes [95] in cluster bean.

Deep sequencing has been used to identify miRNAs in cluster bean, but it generates large amounts of data and requires expertise, making it costly and less feasible for routine applications [96]. In silico approaches that use databases like EST and GSS offer a fast and cost-effective alternative [97]. By utilizing ESTs from the NCBI database, 57 potential miRNA families were identified in the cluster bean with 623 target genes [36]. Among the identified miRNAs, miR837, miR838, and miR5021 have a role in plant tolerance of oxidative stress. On the other hand, miR5021, miR5658, and miR396 were predicted to target MADS floral homeotic protein that play a crucial role in flower development. Notably, many of these target genes were found to be involved in diverse metabolic processes. miRNAs can interact with transcription factors (TFs) to influence plant's ability for stress tolerance. They also found some correlation between miRNA and TFs. They used plant transcription factor database to identify transcription factors in the EST sequences of cluster bean. The study led to identification of many TFs - the majority of which belong to MIKC_MADS family protein basic leucine-zipper and C3H family proteins. For example, miR5021 and miR5658 target MADS-BOX, known for its important role in leaf and flower development. In addition, miR5658 also targets TFs related to heat and drought stress, and regulates growth and reproduction processes. MiR838 targets NAC TF, which plays an important role in maintaining root architecture. The presence of miR169-specific amplicons in 'HG 2-20' and 'RGC 471' strongly suggests their involvement in disease

How to cite this article:

. P, Jangra S, Chaudhary V, et al. (May 20, 2026) Biotechnological Interventions in Unravelling the Genetic Treasure of an Orphan Crop, Cluster Bean (*Cyamopsis tetragonoloba* L.). Curues J. Agri. Food Sci 2 : es44497-025-00069-2. DOI <https://doi.org/10.7759/s44497-025-00069-2>

resistance in cluster bean. These findings provide valuable insights into the role of miRNAs in regulating biotic stress tolerance in cluster bean. However, further experiments can confirm the functional roles of miR169 and its interplay with TFs. Understanding these regulatory mechanisms can potentially lead to the development of cluster bean varieties with enhanced biotic stress resistance, contributing to sustainable agriculture and food security [98]. Recently, Sahu et al. [37] predicted the role of cluster bean miRNAs (cb-miRs) in human and cattle health. They predicted that cb-miRs work similarly to human and cattle miRNAs.

Overall, current evidence suggests that miRNAs may play an important regulatory role in modulating key steps of galactomannan biosynthesis, particularly through targeting enzymes such as ManS and UGE. The putative roles of miRNAs in the regulation of the galactomannan biosynthetic framework are represented in Figure 3. This provides a more comprehensive understanding of how metabolic and regulatory networks coordinate guar gum accumulation during seed development. However, most of these miRNA-target interactions are based on computational predictions or expression correlations, and robust experimental validation using approaches such as degradome analysis, gene knockdown, or overexpression studies remains limited in cluster bean. Table 5 presents top miRNA families identified in cluster bean and their functions.

miRNA family	Expression site	Putative function in cluster bean
miR159	Most abundant in seeds	Regulation of galactomannan pathway
miR166	Moderately expressed throughout the plant	Regulation of galactomannan pathway
miR156	Moderately expressed throughout the plant	Development regulation
miR319	Both vegetative and reproductive tissues (leaf, root, stem, seed, and bud)	Regulation of galactomannan pathway
miR167	Moderately expressed throughout the plant	Stress response
miR168	Reproductive tissues (seed and bud)	Stress response
Ct-miR3130	Seed	Regulation of galactomannan pathway
Ct-miR3135	Vegetative tissues	Regulation of galactomannan pathway
Ct-miR3157	Vegetative tissues	Regulation of galactomannan pathway
miR172	-	Flower development
miR2606	-	Stress response

TABLE 5: Top miRNA families identified in cluster bean and their functions.

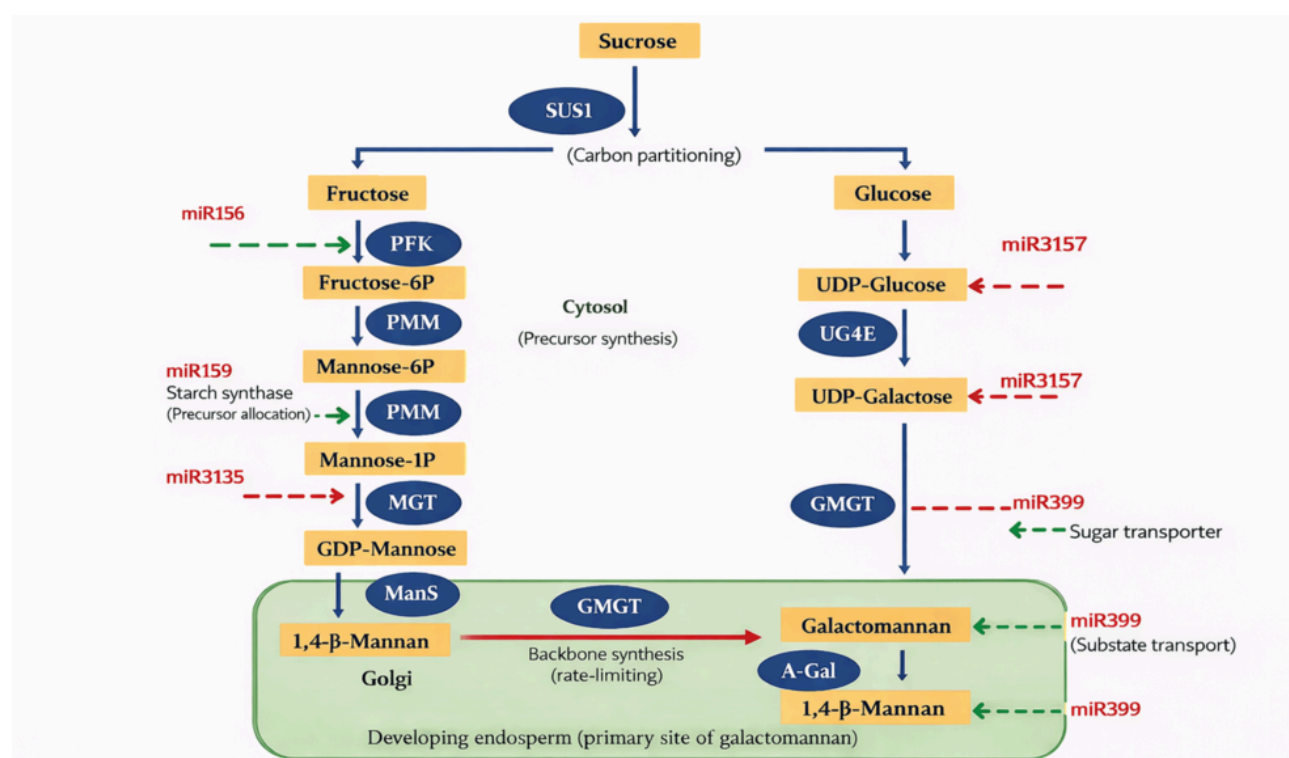


FIGURE 3: Galactomannan biosynthesis and miRNA-mediated regulation in guar.

Sucrose is cleaved by SUS1 into glucose and fructose, which feed into coordinated pathways generating GDP-mannose (via PFK, PMI, PMM, MGT) and UDP-galactose (via UG4E). Mannan synthase (ManS) forms the mannan backbone, and galactomannan galactosyltransferase (GMGT) catalyzes polymer assembly in the Golgi. Transcriptomic studies show upregulation of key genes (ManS, GMGT, PMI, PMM, MGT, UG4E) during 30–40 days after flowering, coinciding with peak gum accumulation [75,81]. MiRNA regulation includes validated interactions (Ct-miR3135–ManS; Ct-miR3157–UG4E) [35] and predicted regulators (e.g., miR156, miR159, miR319, miR399).

GDP, Guanosine Diphosphate; MGT, Mannose-1-Phosphate Guanyltransferase; PFK, Phosphofructokinase; PMI, Phosphomannose Isomerase; PMM, Phosphomannomutase; UDP, Uridine Diphosphate

Decoding the guar gum synthesis pathway

Cluster bean is a galactomannan-rich legume that accumulates guar gum within the seed endosperm. Galactomannan belongs to a polysaccharide group comprising a backbone of mannose-bearing galactofuran side chains, with variable numbers of side chains. The pathway begins with the reversible cleavage of sucrose into glucose and fructose by sucrose synthase (SUS1). These hexose sugars are subsequently funneled into two major branches. In the mannose branch, fructose is converted into mannose-6-phosphate through sequential enzymatic reactions involving phosphofructokinase (PFK) and phosphomannose isomerase (PMI), followed by conversion to mannose-1-phosphate by phosphomannomutase (PMM). Mannose-1-phosphate is then converted to GDP-mannose by mannose-1-phosphate guanyltransferase (MGT), which serves as the substrate for mannan synthase (ManS) to generate the β-1,4-mannan backbone.

In parallel, the glucose-derived pathway leads to the formation of UDP-glucose, which is subsequently converted to UDP-galactose by UDP-glucose 4-epimerase (UG4E). In the Golgi apparatus, galactomannan galactosyltransferase (GMGT) catalyzes the transfer of galactose residues onto the mannan backbone, resulting in the formation of galactomannan. The coordinated activity of ManS and GMGT is therefore central to guar gum biosynthesis.

How to cite this article:

. P, Jangra S, Chaudhary V, et al. (May 20, 2026) Biotechnological Interventions in Unravelling the Genetic Treasure of an Orphan Crop, Cluster Bean (*Cyamopsis tetragonoloba* L.). Curues J. Agri. Food Sci 2 : es44497-025-00069-2. DOI <https://doi.org/10.7759/s44497-025-00069-2>

The schematic representation of this pathway (Figure 3) integrates cluster bean-specific transcriptomic evidence with established knowledge from general legume carbohydrate metabolism models. Multiple RNA-Seq studies have consistently shown stage-specific upregulation of key biosynthetic genes, including ManS, GMGT1, GDPMP, PMM, and MAN, particularly during 30 to 40 days after flowering, coinciding with peak galactomannan accumulation [75,81,99,100]. In addition, qRT-PCR validation has confirmed the differential expression of several of these genes across contrasting genotypes and developmental stages, supporting their role in guar gum biosynthesis [81,100]. However, while core enzymatic steps such as ManS- and GMGT-mediated polymer assembly are well supported by expression and validation data [100,101], several upstream metabolic and regulatory components of the pathway remain inferred from transcriptomic analyses, homology, or related legume systems.

Seed development marks a pivotal phase where galactomannan biosynthesis becomes the predominant metabolic activity within the guar seed endosperm. Galactomannan synthesis in the seed's endosperm is catalyzed by the action of two membrane-bound enzymes: ManS and GMGT [102-104]. ManS facilitates the incremental transfer of mannosyl residues to the growing mannan chain, while GMGT plays an important role in transferring galactose residues to specific galactosyl acceptor mannose residues, in proximity to the growing mannan chain end [105].

The initial identification of the ManS gene in guar occurred within the cellulose synthase-like A (CSLA) gene family. This gene family, known for its association with cellulose synthesis, particularly the glucomannan or mannan backbone, was further examined *in vitro*. This experiment revealed that the synthesis activity of CSLA proteins was involved in the production of mannan or glucomannan [106]. Subsequent investigations using CSLA mutants in *Arabidopsis* confirmed the pivotal role of CSLA proteins in the *in vivo* biosynthesis of glucomannan [107]. Through a series of experiments, it was observed that significant accumulation of ManS, GMGT, and cellulose synthase in endosperm takes place from 30 to 40 days after flowering (DAF) in the guar seed endosperm [61,99,106].

To investigate the genes involved in galactomannan synthesis, Hu et al. [99] performed RNA sequencing on embryos and endosperms collected from guar pods at 30 and 40 days after flowering (DAF), and identified 2535 and 2724 DEGs in the embryo and endosperm, respectively [99]. This implies that the processes controlling the guar endosperm and the synthesis of guar gum are complex. Several genes, like ManS, GMGT1, mannose 1-phosphate guanylyl transferase (GDPMP), phosphomannomutase (PMM), and beta-mannosidase (MAN), were highly expressed in the endosperm from 30 to 40 DAF, suggesting their critical role in galactomannan synthesis. Chaudhary et al. [75] also studied the galactomannan biosynthesis pathway by comparing gene expression patterns in young pods, mature pods, and young leaves of 'HG365' and 'HG870' guar cultivars. Major genes involved in galactomannan biosynthesis, including Cellulose synthase D1 (CS D1) and GAUT-like, were identified. The CS transcripts were predominantly present in the leaf samples, while the expression of GAUT-like gene increased from 65% to 90% during 10 to 50 days after anthesis (DAA). In addition, galactomannan content progressively rises in 'HG870' (7.035-41.2%) and 'HG365' (12.98-20.66%) during 10 to 50 DAA, with a maximum accumulation observed in mature and dry seeds and exhibiting a 3.8-to-7.1-fold increase. Altogether, these results indicate that the expression of GAUT-like genes highly correlates with the accumulation of galactomannan in developing seeds. Similarly, Sharma et al. [100] compared transcriptomes of two guar varieties, 'RGC 936' (high) and 'M83' (low), with high contrasting gum content. The transcriptomes from pod tissues were analyzed at 25, 39, and 50 DAF. The transcripts related to gum content showed the highest expression at 39 DAF. Additionally, WRKY TF was found to be co-expressed with key genes involved in galactomannan synthesis, and its role can be explored for generating high gum content varieties in guar. Moreover, 691 and 188 candidate unigenes involved in galactomannan biosynthesis were identified and analyzed from both varieties. Another RNA-Seq analysis of immature seeds from two guar genotypes, 'HGS563' (gum type) and 'Pusa Navbahar' (PNB, vegetable type), identified 4,938 DEGs [81]. Out of these, 2,241 genes were upregulated, and 2,587 genes were downregulated in the 'HGS563' genotype. Some of the up-regulated genes were identified to play role in galactomannan synthesis and cell wall storage polysaccharide. Additional up-regulated genes were involved in biosynthesis of galactomannan and carotenoid, wax metabolism, terpenoid, mannose, and galactose nucleotide sugars. In both genotypes, major nucleotide sugar was found to be mannose and galactose. qRT-PCR results were in concordance with transcriptomic data and confirmed the higher expression of sugar accumulation and galactomannan synthesis genes in HG563 than in PNB. As discussed earlier in the miRNA section, various microRNAs

How to cite this article:

. P, Jangra S, Chaudhary V, et al. (May 20, 2026) Biotechnological Interventions in Unravelling the Genetic Treasure of an Orphan Crop, Cluster Bean (*Cyamopsis tetragonoloba* L.). *Curues J. Agri. Food Sci* 2 : es44497-025-00069-2. DOI <https://doi.org/10.7759/s44497-025-00069-2>

(miRNAs) also play significant regulatory roles in galactomannan biosynthesis [34,36,41]. Gaikwad et al. [31] further identified 68,641 transcripts from a reference-based transcriptome dataset, including six distinct tissues of cluster bean, i.e. flower, pod at 25 DAF, pod at 39 DAF, pod at 50 DAF, shoot, and leaf. Expression analysis indicated a pod-specific up-regulation of genes that encode enzymes for Mannose-1-phosphate guanyl transferase (MGT), Glucomannan 4-beta-mannosyl transferase (ManS), Mannose-6-phosphate isomerase (PMI), Phosphofructokinase (PFK), and Phosphomannomutase (PMM), orchestrating the sequential conversion of fructose into 1,4- β mannan. Additionally, genes involved in the glucose to UDP-Galactose conversion process, including UTP-Glucose-1-phosphate uridyl transferase and UDP-Glucose-4-epimerase (UG4E), were also up-regulated during various pod developmental stages. The pivotal enzyme GMGT, catalysing the final step of synthesis of galactomannan, also exhibited up-regulation in pod tissues. These studies collectively contribute to a deeper understanding of guar gum synthesis and the key genes involved in gum synthesis and accumulation in the guar seeds.

Although these studies have identified candidate genes and regulatory pathways involved in galactomannan biosynthesis, their functional validation through gene editing, knockdown, or overexpression studies remains limited in cluster bean.

Functional genomics in cluster bean

Functional genomics approaches have emerged as powerful tools for understanding gene function and enabling targeted crop improvement. Among these, RNA interference (RNAi) has been successfully employed for both disease management and trait modification. Host-induced gene silencing is an RNAi-based, promising approach that has emerged as a widely used tool for engineering crop varieties that withstand a broad spectrum of environmental challenges. In cluster bean, for the first time, RNAi approach has been explored to control *Alternaria cyamopsidis* blight/leaf spot caused by *Alternaria cucumerina* var. *cyamopsidis* in the HG2-20 variety of cluster bean [38]. Researchers silenced the AcCHS7 gene and reported a delay in disease progression by damaging hyphae, reducing mycelium growth to control *Alternaria cyamopsidis* blight of guar crop. Delayed disease progression and increased survival rate may provide guar farmers extra time to diagnose and treat the disease, hence minimizing yield losses.

In another study, RNAi demonstrated the effect of silencing *C. tetragonoloba* *BIG SEEDS LIKE* (*CtBSL*) gene on seed yield and important agronomic traits [39]. They analyzed two independent T4 transgenic lines, BSL12-25-15-31-3 and BSL70-21-25-4-16, via pCMK*CtBSL*-mediated post-transcriptional *CtBSL* silencing approach. They found upregulation of following genes *CtCYCA2;4*, *CtCYCD3;2*, *CtCDKE-1*, *CtCYCT1;5* (Genes related to cell division), *CtH2A*, *CtH2B*, *CtH3*, *CtH4*, *CtERF*, *CtWRKY43*, *CtGRF5* and *CtGIF1* (Genes related to growth regulation) which contributed to enhanced cell proliferation and final seed size which was further positively correlated to phytochemical biosynthesis and their anti-diabetic, anti-Advanced glycation end products (AGEs) and lipase inhibitory potential against α -glucosidase, α -amylase, AGEs and lipase enzymes respectively. Thereby, silencing of *CtBSL* improved the crop yield and aided in therapeutic treatments.

Verma et al. [101] exercised RNAi for downregulating the GMGT, which adds galactose to the mannose backbone chain in guar variety RGC-936, using agrobacterium-mediated transformation. RT-qPCR analysis showed that silencing reduced GMGT levels in the seed endosperm 35 days post-flowering, thereby opening opportunities for gene characterization and improvement of agronomic traits.

Beyond RNAi, genome editing technologies have been utilized to expand the functional genomics toolkit in guar. Notably, the development of a highly efficient CRISPR/Cas9-based protoplast editing system represents the first report of targeted genome editing in guar. Although this system is currently limited to transient assays, it establishes a critical platform for rapid gene function analysis and targeted genome modification.

Overall, functional genomics approaches, particularly RNAi and CRISPR/Cas9, provide valuable tools for dissecting gene function and accelerating crop improvement in cluster bean. However, their application remains in early stages, and further efforts are required to develop stable transformation systems and integrate these tools into practical breeding programs.

How to cite this article:

. P, Jangra S, Chaudhary V, et al. (May 20, 2026) Biotechnological Interventions in Unravelling the Genetic Treasure of an Orphan Crop, Cluster Bean (*Cyamopsis tetragonoloba* L.). *Curues J. Agri. Food Sci* 2 : es44497-025-00069-2. DOI <https://doi.org/10.7759/s44497-025-00069-2>

Genetic resources and genomic databases in cluster bean

Over the past few years, significant research has been conducted towards advancing cluster improvement. In this regard, several resources and databases have been established. India, being the primary exporter of the crop, holds a major germplasm collection at the National Bureau of Plant Genetic Resources (NBPGR) and Central Arid Zone Research Institute (CAZRI), collectively conserving >4,000 accessions. Additional collections are available at the United States Department of Agriculture, National Agricultural Research Centre (NARC, Islamabad), and the Institute of Plant Industry, Russia. These resources capture variations for gum content, yield, and stress tolerance. Among the different collections available at these centres, 27 have been identified as drought-tolerant while 18 are drought-sensitive [9].

The development of structured mapping populations, diversity panels, and mini-core collections is still in its infancy in cluster bean. The lack of well-defined association panels and biparental populations significantly restricts the identification of stable QTLs and marker-trait associations.

Further, several specialized databases and genomic repositories have been developed to facilitate research and breeding in cluster bean, providing access to genomic, transcriptomic, and molecular marker information. ClustergeneDB (<http://14.139.229.201/clustergenedb/>) is a dedicated database developed for the retrieval of unigenes and microsatellite (SSR) markers derived from transcriptome datasets. It serves as an important resource for marker development and functional genomics studies [18]. cbSIR (cluster bean SNPs and InDels Repository: http://webapp.cabgrid.res.in/clb_ce/index.php) is an online platform that stores cultivar-specific SNPs and InDels, along with their functional annotations. This database supports marker-assisted breeding and trait association studies [33]. CbLncRNAdb (Cluster bean lncRNA database: <http://cabgrid.res.in/cblncrnadb>): A specialized database containing information on long non-coding RNAs (lncRNAs), miRNAs, and SSR-associated regulatory elements, along with their roles in biological processes and stress responses [34]. *C. tetragonoloba* Genomic Atlas/Genome Database (<https://guar.ariam.ru/>) provides access to gene annotations, expression datasets, functional annotations (GO, KEGG), and sequence analysis tools enabling genome-wide exploration and comparative studies.

Despite these advances, limited functional validation and lack of gene-based markers restrict direct deployment in breeding. Strengthening mapping populations, expanding diversity panels, and integrating multi-omics datasets will be essential to translate these resources into improved guar cultivars.

Conclusions

Cluster bean is an economically important legume with global industrial relevance driven by guar gum. Its inherent adaptation to arid environments makes it a promising crop for future climate-resilient agriculture. However, despite this potential, productivity gains remain limited due to a narrow genetic base, insufficient resistant germplasm, and incomplete understanding of the genetic and molecular regulation of key traits. This review highlights how recent advances in genomics, transcriptomics, and molecular biology are beginning to bridge this gap. The availability of reference genomes, high-density molecular markers, and transcriptomic datasets has enabled the identification of candidate genes and pathways underlying important traits. In particular, the galactomannan biosynthesis pathway, involving key enzymes such as ManS, GMGT, PMM, and GDPMP, provides a clear biochemical framework for gum accumulation. Importantly, emerging evidence from miRNA studies demonstrates an additional regulatory layer, where miRNAs modulate genes involved in carbohydrate metabolism, sugar transport, and endosperm development, linking gene regulation directly to phenotypic outcomes. The integration of these findings, as illustrated in Figure 3, provides a systems-level view of guar gum biosynthesis, connecting metabolic pathways with transcriptional and post-transcriptional regulation. Similarly, advances in marker systems (SSRs, SNPs, and InDels), GWAS, and transcriptome-based analyses have enabled the identification of genomic regions and candidate genes associated with yield, stress tolerance, and nodulation. However, these resources remain underutilized, and major QTLs and functionally validated genes are still limited. In addition, many existing studies remain largely descriptive, highlighting the need for more rigorous experimental validation and comparative analysis across studies.

How to cite this article:

. P, Jangra S, Chaudhary V, et al. (May 20, 2026) Biotechnological Interventions in Unravelling the Genetic Treasure of an Orphan Crop, Cluster Bean (*Cyamopsis tetragonoloba* L.). Curues J. Agri. Food Sci 2 : es44497-025-00069-2. DOI <https://doi.org/10.7759/s44497-025-00069-2>

Moving forward, the key challenge lies in translating these genomic insights into breeding outcomes. This requires integrating multi-omics datasets with high-throughput phenotyping and developing structured populations for robust trait mapping. Functional validation of candidate genes and regulatory elements, including miRNAs, will be critical to confirm their roles in gum biosynthesis and stress adaptation. In this context, functional genomics approaches such as RNAi and CRISPR/Cas9 provide a direct link between gene discovery and trait improvement, enabling targeted manipulation of key pathways involved in stress tolerance, yield, and gum biosynthesis. Expanding and systematically characterizing global germplasm, including wild relatives, will be essential to broaden the genetic base and introduce novel alleles for stress resilience and yield improvement. Ultimately, a coordinated framework that integrates genetic resources, molecular markers, regulatory networks, and functional genomics will be required to fully exploit the potential of cluster bean. Despite recent advances, cluster bean remains in a pre-genomic breeding phase compared to major legume crops, highlighting a significant gap between marker discovery and field-level application. Overall, integrating genomics, functional genomics, and advanced breeding strategies will be essential to bridge the gap between molecular discoveries and field-level applications, ultimately enabling the development of high-yielding, stress-resilient, and industrially superior guar cultivars.

Additional Information

Author Contributions

All authors have reviewed the final version to be published and agreed to be accountable for all aspects of the work.

Concept and design: Sumit Jangra, Priti ., Neelam R. Yadav, Ram C. Yadav

Acquisition, analysis, or interpretation of data: Sumit Jangra, Priti ., Vrantika Chaudhary, Rizwana Rehsawla, Aditi Taneja, Jugpreet Singh

Drafting of the manuscript: Sumit Jangra, Priti ., Vrantika Chaudhary, Rizwana Rehsawla, Aditi Taneja, Jugpreet Singh, Neelam R. Yadav, Ram C. Yadav

Supervision: Sumit Jangra, Priti ., Neelam R. Yadav, Ram C. Yadav

Disclosures

Conflicts of interest: In compliance with the ICMJE uniform disclosure form, all authors declare the following:

Payment/services info: All authors have declared that no financial support was received from any organization for the submitted work. **Financial relationships:** All authors have declared that they have no financial relationships at present or within the previous three years with any organizations that might have an interest in the submitted work. **Other relationships:** All authors have declared that there are no other relationships or activities that could appear to have influenced the submitted work.

Data Availability Statements

Data sharing is not applicable. This work is theoretical; no datasets were generated or analyzed.

References

1. Kumar D, Rodge AB: [Status, scope and strategies of arid legumes research in India- A review](#). Journal of Food Legumes. 2024, 25:255-272.
2. Mudgil D, Barak S, Khatkar BS: [Guar gum: processing, properties and food applications—A Review](#). Journal of Food Science and Technology. 2011, 51:409-418. [10.1007/s13197-011-0522-x](#)
3. Hussain Z, Mahajan M: [Hidden potential of cluster bean: An unexploited legume crop for food and nutritional security](#). Vegetos. 2025, [10.1007/S42535-025-01333-8](#)

How to cite this article:

. P, Jangra S, Chaudhary V, et al. (May 20, 2026) Biotechnological Interventions in Unravelling the Genetic Treasure of an Orphan Crop, Cluster Bean (*Cyamopsis tetragonoloba* L.). Curues J. Agri. Food Sci 2 : es44497-025-00069-2. DOI <https://doi.org/10.7759/s44497-025-00069-2>

4. Gautam R, Verma AK, Dwivedi S, Jhang T: [Breeding guar \[*Cyamopsis tetragonoloba* \(L.\) Taub\]: A variety compendium of a multifaceted industrial crop for resource-constrained scenario in India](#). *Industriail Crops and Products*. 2024, 214:118502. [10.1016/J.INDCROP.2024.118502](#)
5. Chauhan JS, Singh KH, Choudhury PR, Singh RK, Mishra JP: [Growth, varietal development and cultivar diversity in seed production chain of cluster bean \(*Cyamopsis tetragonoloba*\) in India](#). *The Indian Journal of Agricultural Sciences*. 2023, 93:811-820. [10.56093/ijas.v93i8.125470](#)
6. Sultan M, Zakir N, Rabbani MA, Shinwari ZK, Shahid Masood M: [Genetic diversity of guar \(*Cyamopsis tetragonoloba*\) landraces from Pakistan based on RAPD markers](#). *Pakistan Journal of Botany*. 2013, 45:865-870.
7. Deepika, Dhingra HR: [Effect of salinity stress on morpho-physiological, biochemical and yield characters of cluster bean \[*Cyamopsis tetragonoloba* \(L.\) Taub.\]](#). *Indian Journal of Plant Physiology*. 2014, 19:393-398. [10.1007/s40502-014-0118-3](#)
8. Puzanskiy RK, Romanyuk DA, Zorin EA, Vishnyakova MA, Zhukov VA: [Metabolomic analysis of drought response in guar \(*Cyamopsis Tetragonoloba* \(L.\) Taub.\) seeds](#). *Agronomy*. 2025, 15:2778. [10.3390/agronomy15122778](#)
9. Vishnyakova MA, Frolova N, Frolov A: [Drought stress response in guar \(*Cyamopsis tetragonoloba* \(L.\) Taub\): Physiological and molecular genetic aspects](#). *Plants*. 2023, 12:3955. [10.3390/plants12233955](#)
10. Sandhu D, Pallete A, Pudussery MV, Grover KK: [Contrasting responses of gar genotypes shed light on multiple component traits of salinity tolerance mechanisms](#). *Agronomy*. 2021, 11:1068. [10.3390/agronomy11061068](#)
11. Gresta F, Mercati F, Santonoceto C, et al.: [Morpho-agronomic and AFLP characterization to explore guar \(*Cyamopsis tetragonoloba* L.\) genotypes for the Mediterranean environment](#). *Industrial Crops and Products*. 2016, 86:23-30. [10.1016/j.indcrop.2016.03.038](#)
12. Gillaspie AG Jr, Pappu HR, Jain RK, Rey MEC, Hopkins MS, Pinnow DL, Morris JB: [Characteristics of a latent potyvirus seedborne in guar and of guar green-sterile virus](#). *Plant Disease*. 1998, 82:765-770. [10.1094/PDIS.1998.82.7.765](#)
13. Jain R, Agrawal K: [Incidence and seed transmission of *Xanthomonas axonopodis* Pv. *Cyamopsidis* in cluster bean](#). *Journal of Agricultural Technology*. 2011, 7:197-205.
14. Gahlot VS, Kumhar D, Kumar D, Kumari N: [Appraisal to efficacy of different bioagents and fungicides against stem rot of cluster bean incited by *Sclerotium rolfsii*](#). *Biological Forum - An International Journal*. 2022, 14:1164-1168.
15. Bagri RK, Goyal SK, Singh J, Priyanka, Chawla N, Kumari M: [Integrated pest management of cluster bean \(*Cyamopsis tetragonoloba* L.\)](#). *The Pharma Innovation*. 2021, 10:679-682. [10.22271/tpi.2021.v10.i3j.5855](#)
16. Kumar S, Meena AK, Kumar M, Rani K: [Evaluation for resistance of cluster bean varieties against *Alternaria* leaf blight in vivo](#). *International Journal of Current Microbiology and Applied Sciences*. 2020, 9:2107-2111. [10.20546/IJCMAS.2020.909.262](#)
17. Santonoceto C, Mauceri A, Lupini A, et al.: [Morpho-agronomic characterization and genetic variability assessment of a guar germplasm collection by a novel SSR panel](#). *Industrial Crops and Products*. 2019, 138:111568. [10.1016/j.indcrop.2019.111568](#)
18. Rawal HC, Kumar S, Amitha Mithra SV, et al.: [High quality unigenes and microsatellite markers from tissue specific transcriptome and development of a database in clusterbean \(*Cyamopsis tetragonoloba*, L. Taub\)](#). *Genes*. 2017, 8:313. [10.3390/genes8110313](#)
19. Malani S, Ravelombola W, Manley A, Pham H: [Genetic diversity and population structure analysis in guar](#). *Plants*. 2024, 13:3183. [10.3390/plants13223183](#)
20. Punia A, Arora P, Yadav R, Chaudhary A: [Optimization and inference of PCR conditions for genetic variability studies of commercially important cluster bean varieties by RAPD analysis](#). *Asia Pacific Journal of Molecular Biology and Biotechnology*. 2009, 17:33-38.
21. Punia A, Yadav R, Arora P, Chaudhury A: [Molecular and morphophysiological characterization of superior cluster bean \(*Cymopsis tetragonoloba*\) varieties](#). *Journal of Crop Science and Biotechnology*. 2009, 12:143-148. [10.1007/s12892-009-0106-8](#)
22. Pathak R, Singh SK, Singh M, Henry A: [Molecular assessment of genetic diversity in cluster bean \(*Cyamopsis tetragonoloba*\) genotypes](#). *Journal of Genetics*. 2010, 89:243-246. [10.1007/s12041-010-0033-y](#)
23. Kuravadi AN, Tiwari PB, Choudhary M, Tripathi SK, Dhugga KS, Gill KS, Singh Randhawa G: [Genetic diversity study of cluster bean \(*Cyamopsis tetragonoloba* \(L.\) Taub\) landraces using RAPD and ISSR markers](#). *International Journal of Advanced Biotechnology and Research*. 2013, 4:460-471.
24. Kumar V, Ram RB, Kumar Rajvanshi SK: [Standardization of DNA isolation and PCR protocols for ISSR analysis in cluster bean \[*Cyamopsis Tetragonoloba* \(L.\) Taub.\] Genotypes](#). *International Journal of Advanced Biotechnology and Research*. 2015, 6:203-210.

How to cite this article:

. P, Jangra S, Chaudhary V, et al. (May 20, 2026) Biotechnological Interventions in Unravelling the Genetic Treasure of an Orphan Crop, Cluster Bean (*Cyamopsis tetragonoloba* L.). *Curues J. Agri. Food Sci 2* : es44497-025-00069-2. DOI <https://doi.org/10.7759/s44497-025-00069-2>

25. Kuravadi NA, Tiwari PB, Tanwar UK, Tripathi SK, Dhugga KS, Gill KS, Singh Randhawa G: [Identification and characterization of EST-SSR markers in cluster bean \(*Cyamopsis* spp.\)](#). Crop Science. 2014, 54:1097-1102. [10.2135/cropsci2013.08.0522](#)
26. Wadhwa N, Aditi, Joshi UN, Yadav NR: [EST-SSRs provide a good measure of genetic diversity for improvement of gum content in cluster bean](#). Proceedings of the Proceedings of International Grassland Congress. 2015, 1-3.
27. Boghara MC, Dhaduk HL, Kumar S, Parekh MJ, Patel NJ, Sharma R: [Genetic divergence, path analysis and molecular diversity analysis in cluster bean \(*Cyamopsis tetragonoloba* L. Taub.\)](#). Industrial Crops and Products. 2016, 89:468-477. [10.1016/j.indcrop.2016.05.049](#)
28. Tribhuvan KU, Amitha Mithra SV, Sharma P, et al.: [Identification of genomic SSRs in cluster bean \(*Cyamopsis tetragonoloba*\) and demonstration of their utility in genetic diversity analysis](#). Industrial Crops and Products. 2019, 133:221-231. [10.1016/J.INDCROP.2019.03.028](#)
29. Kaila T, Chaduvla PK, Rawal HC, et al.: [Chloroplast genome sequence of clusterbean \(*Cyamopsis tetragonoloba* L.\): Genome structure and comparative analysis](#). Genes. 2017, 8:212. [10.3390/genes8090212](#)
30. Grigoreva E, Ulianich P, Ben C, Gentzbittel L, Potokina E: [First insights into the guar \(*Cyamopsis tetragonoloba* \(L.\) Taub.\) genome of the 'Vavilovskij 130' accession, using second and third-generation sequencing technologies](#). Russian Journal of Genetics. 2019, 55:1406-1416. [10.1134/s102279541911005x](#)
31. Gaikwad K, Ramakrishna G, Srivastava H, et al.: [The chromosome-scale genome assembly of cluster bean provides molecular insight into edible gum \(galactomannan\) biosynthesis family genes](#). Scientific Reports. 2023, 13:9941. [10.1038/s41598-023-33762-3](#)
32. Tanwar UK, Pruthi V, Randhawa GS: [RNA-Seq of guar \(*Cyamopsis tetragonoloba*, L. Taub\) leaves: De novo transcriptome assembly, functional annotation and development of genomic resources](#). Frontiers in Plant Science. 2017, 8:91. [10.3389/fpls.2017.00091](#)
33. Sahu S, Sahu TK, Ghosal S, Gaikwad K, Rao AR: [Computational analysis of SNPs and INDELs in cluster bean cultivars involved in multiple trait expression](#). Indian Journal of Genetics and Plant Breeding. 2020, 80:179-185.
34. Sahu S, Rao AR, Pandey J, Gaikwad K, Ghoshal S, Mohapatra T: [Genome-wide identification and characterization of lncRNAs and miRNAs in cluster bean \(*Cyamopsis tetragonoloba*\)](#). Gene. 2018, 667:112-121. [10.1016/j.gene.2018.05.027](#)
35. Tyagi A, Nigam D, Amitha Mithra SV, Solanke AU, Singh NK, Sharma TR, Gaikwad K: [Genome-wide discovery of tissue-specific miRNAs in clusterbean \(*Cyamopsis tetragonoloba*\) indicates their association with galactomannan biosynthesis](#). Plant Biotechnology Journal. 2018, 16:1241-1257. [10.1111/pbi.12866](#)
36. Chaudhary V, Jangra S, Yadav NR: [In silico identification of miRNAs and their targets in cluster bean for their role in development and physiological responses](#). Frontiers in Genetics. 2022, 13:930113. [10.3389/fgene.2022.930113](#)
37. Sahu S, Rao AR, Sahu TK, Pandey J, Varshney S, Kumar A, Gaikwad K: [Predictive role of cluster bean \(*Cyamopsis tetragonoloba*\) derived miRNAs in human and cattle health](#). Genes. 2024, 15:448. [10.3390/genes15040448](#)
38. Thakur O, Prasad R: [Engineering resistance to *Alternaria cyamopsidis* by RNAi mediated gene silencing of chitin synthase export chaperone CH57 in guar](#). Physiological and Molecular Plant Pathology. 2020, 112:101541. [10.1016/j.pmpp.2020.101541](#)
39. Thakur O, Prasad R: [Increased seed size mediated by *Cyamopsis tetragonoloba* big SEEDS LIKE silencing is positively co-related to galactomannan content phytochemical biosynthesis and anti-diabetic potential of guar \[PREPRINT\]](#). 2021, [10.21203/RS.3.RS-306592/V1](#)
40. Ghosh PK, Biswas S, Malaker R, Pham H, Septiningsih EM, Ravelombola W: [Optimization of CRISPR/Cas9-mediated CtPDS knockout in guar protoplasts](#). The Plant Genome. 2026, 19:e70177. [10.1002/tpg2.70177](#)
41. Kumar R, Bohra A, Pandey AK, Pandey MK, Kumar A: [Metabolomics for plant improvement: Status and prospects](#). Frontiers in Plant Science. 2017, 8:1302. [10.3389/fpls.2017.01302](#)
42. Ajit P, More V, Sukhadev K, Parimal V, Bhausaheb G: [Molecular characterization of cluster bean \(*Cyamopsis tetragonoloba*\) cultivars using PCR-based molecular markers](#). International Journal of Advanced Biotechnology and Research. 2013, 4:158-166.
43. Ghorpade B, Savant A, Nalge S, Gunjal P: [Molecular characterization of cluster bean \(*Cyamopsis tetragonoloba* L. Taub\) using RAPD markers](#). International Journal of Innovative Research in Science, Engineering and Technology. 2018, 7:9087-9093.
44. Sharma A, Sharma P, Singh YP: [Phytochemical and genetic analysis of *Cyamopsis tetragonoloba* by RAPD, ISSR and HPLC](#). Biochemical and Cellular Archives. 2012, 12:31-40.

How to cite this article:

. P, Jangra S, Chaudhary V, et al. (May 20, 2026) Biotechnological Interventions in Unravelling the Genetic Treasure of an Orphan Crop, Cluster Bean (*Cyamopsis tetragonoloba* L.). Curues J. Agri. Food Sci 2 : es44497-025-00069-2. DOI <https://doi.org/10.7759/s44497-025-00069-2>

45. Sharma P, Sharma V, Kumar V: [Genetic diversity analysis of cluster bean \[*Cyamopsis tetragonoloba* \(L.\) Taub\] genotypes using RAPD and ISSR markers](#). Journal of Agricultural Science and Technology. 2014, 16:433-443.
46. Kumar S, Joshi UN, Sangwan S, Yadav R, Singh JV, Saini ML: [Biomolecular characterization of guar \(*Cyamopsis tetragonoloba*\) genotypes along with wild species, *C. serrata* and *C. senegalensis*](#). Plant Systematics and Evolution. 2014, 301:1249-1262. [10.1007/s00606-014-1148-z](#)
47. Kalaskar SR, Acharya S, Patel JB, Sheikh WA, Rathod AH, Shinde AS: [Genetic diversity assessment in clusterbean \(*Cyamopsis Tetragonoloba* \(L\) Taub.\) by RAPD markers](#). Journal of Food Legumes. 2014, 27:92-94.
48. Ansari A, Singh Sikarwar P, Lade S, Kumar Yadav H, Ranade SA: [Genetic diversity clusters in germplasm of cluster bean \(*Cyamopsis tetragonoloba* L., Taub\), an important food and an industrial legume crop](#). Journal of Agricultural Science and Technology. 2016, 18:1393-1406.
49. Kumar V, Rajvanshi SK, Sharma AK: [Studies on genetic diversity and variability in cluster bean \[*Cyamopsis tetragonoloba* \(L.\) Taub.\] genotypes through morphological and molecular characterization](#). Vegetable Science. 2024, 51:220-227. [10.61180/vegsci.2024.v51.i2.03](#)
50. Bajaj A, Pahuja SK: [QTL mapping of bacterial blight resistance in cluster bean by molecular markers](#). Forage Research. 2024, 50:249-257.
51. Dadheech R, Sharma R, Mahla HR, Bhatt RK: [Morphological and molecular diversity analysis in cluster bean \[*Cyamopsis tetragonoloba* \(L.\) Taub\]](#). International Journal of Pharmacy and Biological Sciences. 2019, 9:378-387.
52. Sharma P, Kumar V, Venkat Raman K, Tiwari K: [A set of SCAR markers in cluster bean \(*Cyamopsis tetragonoloba* L. Taub\) genotypes](#). Advances in Bioscience and Biotechnology. 2014, 5:131-141. [10.4236/abb.2014.52017](#)
53. Deepashree G, Raut N, Gasti VD, et al.: [Molecular and morphological diversity among the cluster bean \[*Cyamopsis tetragonoloba* \(L.\) Taub.\] genotypes](#). Genetic Resources and Crop Evolution. 2022, 70:159-168. [10.1007/s10722-022-01419-1](#)
54. Kumar S, Parekh MJ, Patel CB, et al.: [Development and validation of EST-derived SSR markers and diversity analysis in cluster bean \(*Cyamopsis tetragonoloba*\)](#). Journal of Plant Biochemistry and Biotechnology. 2015, 25:263-269. [10.1007/S13562-015-0337-3](#)
55. Aditi: [EST-SSR and RGA polymorphism for diversity analysis and bacterial leaf blight in cluster bean \[*Cyamopsis tetragonoloba* \(L.\) Taub.\]](#). CCS Haryana Agricultural University, Hisar; 2018.
56. Kaur A: [Molecular marker analysis for bacterial blight resistance in Cluster bean \(*Cyamopsis tetragonoloba* \(L.\) Taub.\)](#). CCS Haryana Agricultural University, Hisar; 2014.
57. Priti, Panchta R, Yadav RC, Pahuja SK, Yadav NR: [Linkage mapping and identification of quantitative trait loci associated with bacterial leaf blight resistance and gum content in cluster bean using the interspecific population \(HG563 × *Cyamopsis serrata*\)](#). Genetic Resources and Crop Evolution. 2022, 70:1501-1516. [10.1007/s10722-022-01517-0](#)
58. Adbhai A, Talati JG, Kumar S, Sakure A, Acharya RR, Patel NJ: [Study of galactomannan content, gum viscosity and rheological properties and diversity analysis through biochemical traits and simple sequence repeat \(SSR\) markers in cluster bean](#). Genetic Resources and Crop Evolution. 2025, 72:6361-6373. [10.1007/s10722-025-02404-0](#)
59. Kumar S, Palve AS, Patel SK, Selvanayagam S, Sharma R, Rathore A: [Development of genomic microsatellite markers in cluster bean using next-generation DNA sequencing and their utility in diversity analysis](#). Current Plant Biology. 2020, 21:100134. [10.1016/j.cpb.2019.100134](#)
60. Grigoreva E, Barbitoff Y, Changalidi A, et al.: [Development of SNP set for the marker-assisted selection of guar \(*Cyamopsis Tetragonoloba* \(L.\) Taub.\) based on a custom reference genome assembly](#). Plants. 2021, 10:2063. [10.3390/plants10102063](#)
61. Naoumkina M, Torres-Jerez I, Allen S, He J, Zhao PX, Dixon RA, May GD: [Analysis of cDNA libraries from developing seeds of guar \(*Cyamopsis tetragonoloba*\(L.\) Taub\)](#). BMC Plant Biology. 2007, 7:62. [10.1186/1471-2229-7-62](#)
62. Thakur O, Randhawa GS: [Identification and characterization of SSR, SNP and InDel molecular markers from RNA-Seq data of guar \(*Cyamopsis tetragonoloba*, L. Taub.\) roots](#). BMC Genomics. 2018, 19:951. [10.1186/s12864-018-5205-9](#)
63. Al-Qurainy F, Alshameri A, Gaafar AR, et al.: [Comprehensive stress-based de novo transcriptome assembly and annotation of guar \(*Cyamopsis Tetragonoloba* \(L\) Taub.\): an important industrial and forage crop](#). International Journal of Genomics. 2019, 2019:7295859. [10.1155/2019/7295859](#)
64. Ansari MA, Bano N, Kumar A, et al.: [Comparative transcriptomic analysis and antioxidant defense mechanisms in clusterbean \(*Cyamopsis tetragonoloba* \(L.\) Taub.\) genotypes with contrasting drought tolerance](#). Functional & Integrative Genomics. 2022,

How to cite this article:

. P, Jangra S, Chaudhary V, et al. (May 20, 2026) Biotechnological Interventions in Unravelling the Genetic Treasure of an Orphan Crop, Cluster Bean (*Cyamopsis tetragonoloba* L.). Curues J. Agri. Food Sci 2 : es44497-025-00069-2. DOI <https://doi.org/10.7759/s44497-025-00069-2>

22:625-642. [10.1007/s10142-022-00860-w](https://doi.org/10.1007/s10142-022-00860-w)

65. Reddy BM, Anthony Johnson AM, Jagadeesh Kumar N, Venkatesh B, Jayamma N, Pandurangaiah M, Sudhakar C: [De novo transcriptome analysis of drought-adapted cluster bean \(cultivar RGC-1025\) reveals the wax regulatory genes involved in drought resistance](#). *Frontiers in Plant Science*. 2022, 13:868142. [10.3389/fpls.2022.868142](https://doi.org/10.3389/fpls.2022.868142)
66. Arkhimandritova S, Shavarda A, Potokina E: [Key metabolites associated with the onset of flowering of guar genotypes \(Cyamopsis tetragonoloba \(L.\) Taub\)](#). *BMC Plant Biology*. 2020, 20:291. [10.1186/s12870-020-02498-x](https://doi.org/10.1186/s12870-020-02498-x)
67. Shrestha R, Adams CB, Ravelombola W, MacMillan J, Trostle C, Ale S, Hinson P: [Exploring phenotypic variation and associations in root nodulation, morphological, and growth character traits among 50 guar genotypes](#). *Industrial Crops and Products*. 2021, 171:113831. [10.1016/j.indcrop.2021.113831](https://doi.org/10.1016/j.indcrop.2021.113831)
68. Cheng Y, Zhang L, Qi J, Zhang L: [Complete chloroplast genome sequence of Hibiscus cannabinus and comparative analysis of the Malvaceae family](#). *Frontiers in Genetics*. 2020, 11:227. [10.3389/fgene.2020.00227](https://doi.org/10.3389/fgene.2020.00227)
69. Wicke S, Schneeweiss GM, dePamphilis CW, Müller KF, Quandt D: [The evolution of the plastid chromosome in land plants: gene content, gene order, gene function](#). *Plant Molecular Biology*. 2011, 76:273-297. [10.1007/s11103-011-9762-4](https://doi.org/10.1007/s11103-011-9762-4)
70. Sohoo MS, Gill KS: [Asynapsis in cluster bean \(Cyamopsis tetragonoloba \(L.\) Taub.\)](#). *Theoretical and Applied Genetics*. 1975, 46:411-412. [10.1007/bf00281146](https://doi.org/10.1007/bf00281146)
71. Sarbhoy RK: [Spontaneous occurrence of cytomixis and syndiploidy in Cyamopsis tetragonoloba \(L.\) Taub](#). *Cytologia*. 1980, 45:375-379.
72. Patil CG: [Nuclear DNA amount variation in Cyamopsis D.C. \(Fabaceae\)](#). *Cytologia*. 2004, 69:59-62. [10.1508/cytologia.69.59](https://doi.org/10.1508/cytologia.69.59)
73. Tyagi A, Sandhya, Sharma P, et al.: [The genome size of clusterbean \(Cyamopsis tetragonoloba\) is significantly smaller compared to its wild relatives as estimated by flow cytometry](#). *Gene*. 2019, 707:205-211. [10.1016/j.gene.2019.02.090](https://doi.org/10.1016/j.gene.2019.02.090)
74. Li J-H, Li M-J, Li W-L, et al.: [Leguminous industrial crop guar \(Cyamopsis Tetragonoloba\): The chromosome-level reference genome de novo assembly](#). *Industrial Crops and Products*. 2024, 216:118748. [10.1016/j.indcrop.2024.118748](https://doi.org/10.1016/j.indcrop.2024.118748)
75. Chaudhury A, Kaila T, Gaikwad K: [Elucidation of galactomannan biosynthesis pathway genes through transcriptome sequencing of seeds collected at different developmental stages of commercially important Indian varieties of cluster bean \(Cyamopsis Tetragonoloba L.\)](#). *Scientific Reports*. 2019, 9:11539. [10.1038/s41598-019-48072-w](https://doi.org/10.1038/s41598-019-48072-w)
76. Grigoreva E, Tkachenko A, Arkhimandritova S, et al.: [Identification of key metabolic pathways and biomarkers underlying flowering time of guar \(Cyamopsis tetragonoloba \(L.\) Taub.\) via integrated transcriptome-metabolome analysis](#). *Genes*. 2021, 12:952. [10.3390/genes12070952](https://doi.org/10.3390/genes12070952)
77. Alshameri A, Al-Qurainy F, Gaafar AR, Khan S, Nadeem M, Alansi S: [Identification of heat-responsive genes in guar \[Cyamopsis Tetragonoloba \(L.\) Taub\]](#). *International Journal of Genomics*. 2020, 2020:3126592. [10.1155/2020/3126592](https://doi.org/10.1155/2020/3126592)
78. Alshameri A, Al-Qurainy F, Gaafar AR, et al.: [Identification of differentially expressed drought-responsive genes in guar \[Cyamopsis Tetragonoloba \(L.\) Taub\]](#). *International Journal of Genomics*. 2020, 2020:4147615. [10.1155/2020/4147615](https://doi.org/10.1155/2020/4147615)
79. Ashraf MY, Akhtar K, Sarwar G, Ashraf M: [Role of the rooting system in salt tolerance potential of different guar accessions](#). *Agronomy for Sustainable Development*. 2005, 25:243-249. [10.1051/agro:2005019](https://doi.org/10.1051/agro:2005019)
80. Acharya BR, Sandhu D, Dueñas C, Ferreira JFS, Grover KK: [Deciphering molecular mechanisms involved in salinity tolerance in guar \(Cyamopsis Tetragonoloba \(L.\) Taub.\) using transcriptome analyses](#). *Plants*. 2022, 11:291. [10.3390/plants11030291](https://doi.org/10.3390/plants11030291)
81. Rajaprakasam S, Rahman H, Karunakaran S, et al.: [Comparative transcriptome and metabolome profiling in the maturing seeds of contrasting cluster bean \(Cyamopsis tetragonoloba L. Taub\) cultivars identified key molecular variations leading to increased gum accumulation](#). *Gene*. 2021, 791:145727. [10.1016/j.gene.2021.145727](https://doi.org/10.1016/j.gene.2021.145727)
82. Abid G, Sassi K, Muhovski Y, Jacquemin J-M, Minget D, Tarchoun N, Baudoin J-P: [Comparative expression and cellular localization of myo-inositol phosphate synthase \(MIPS\) in the wild type and in an EMS mutant during common bean \(Phaseolus vulgaris L.\) seed development](#). *Plant Molecular Biology Reporter*. 2011, 30:780-793. [10.1007/s11105-011-0377-3](https://doi.org/10.1007/s11105-011-0377-3)
83. Sharma S, Kumar M, Sircar D, Prasad R: [Metabolic profiling and biomarkers identification in cluster bean under drought stress using GC-MS technique](#). *Metabolomics*. 2024, 20:80. [10.1007/s11306-024-02143-w](https://doi.org/10.1007/s11306-024-02143-w)
84. Axtell MJ, Bowman JL: [Evolution of plant microRNAs and their targets](#). *Trends in Plant Science*. 2008, 13:343-349. [10.1016/j.tplants.2008.03.009](https://doi.org/10.1016/j.tplants.2008.03.009)

How to cite this article:

. P, Jangra S, Chaudhary V, et al. (May 20, 2026) Biotechnological Interventions in Unravelling the Genetic Treasure of an Orphan Crop, Cluster Bean (*Cyamopsis tetragonoloba* L.). *Curues J. Agri. Food Sci 2* : es44497-025-00069-2. DOI <https://doi.org/10.7759/s44497-025-00069-2>

85. Li C, Zhang B: [MicroRNAs in control of plant development](#). Journal of Cellular Physiology. 2016, 231:303-313. [10.1002/jcp.25125](#)
86. Chaudhary V, Jangra S, Yadav NR: [Nanotechnology based approaches for detection and delivery of microRNA in healthcare and crop protection](#). Journal of Nanobiotechnology. 2018, 16:40. [10.1186/s12951-018-0368-8](#)
87. Jangra S, Chaudhary V, Yadav NR: [Transcription factors and microRNA interplay: a new strategy for crop improvement](#). Transcriptional and Post-transcriptional Regulation. Ghedira K (ed): IntechOpen, London; 2018. 103-123. [10.5772/intechopen.75942](#)
88. Chen X: [MicroRNA as a translational repressor of APETALA2 in Arabidopsis flower development](#). Science. 2003, 303:2022-2025. [10.1126/science.1088060](#)
89. Waheed S, Zeng L: [The critical role of miRNAs in regulation of flowering time and flower development](#). Genes. 2020, 11:319. [10.3390/genes11030319](#)
90. Liao L, Xie B, Guan P, Jiang N, Cui J: [New insight into the molecular mechanism of miR482/2118 during plant resistance to pathogens](#). Frontiers in Plant Science. 2022, 13:1026762. [10.3389/fpls.2022.1026762](#)
91. Zhang Y, Waseem M, Zeng Z, et al.: [MicroRNA482/2118, a miRNA superfamily essential for both disease resistance and plant development](#). New Phytologist. 2022, 233:2047-2057. [10.1111/nph.17853](#)
92. Li T, Gonzalez N, Inzé D, Dubois M: [Emerging connections between small RNAs and phytohormones](#). Trends in Plant Science. 2020, 25:912-929. [10.1016/J.TPLANTS.2020.04.004](#)
93. Rhoades MW, Reinhart BJ, Lim LP, Burge CB, Bartel B, Bartel DP: [Prediction of plant microRNA targets](#). Cell. 2002, 110:513-520. [10.1016/S0092-8674\(02\)00863-2](#)
94. Rajagopalan R, Vaucheret H, Trejo J, Bartel DP: [A diverse and evolutionarily fluid set of microRNAs in Arabidopsis thaliana](#). Genes & Development. 2006, 20:3407-3425. [10.1101/gad.1476406](#)
95. Vidal EA, Moyano TC, Krouk G, et al.: [Integrated RNA-seq and sRNA-seq analysis identifies novel nitrate-responsive genes in Arabidopsis thaliana roots](#). BMC Genomics. 2013, 14:701. [10.1186/1471-2164-14-701](#)
96. Gasparis S, Yanushevskaya Y, Nadolska-Orczyk A: [Bioinformatic identification and expression analysis of new microRNAs from wheat \(Triticum aestivum L.\)](#). Acta Physiologiae Plantarum. 2017, 39:236. [10.1007/s11738-017-2530-6](#)
97. Vivek AT: [In silico identification and characterization of microRNAs based on EST and GSS in orphan legume crop, Lens culinaris medik. \(Lentil\)](#). Agri Gene. 2018, 8:45-56. [10.1016/j.aggene.2018.05.003](#)
98. Šečić E, Kogel KH, Ladera-Carmona MJ: [Biotic stress-associated microRNA families in plants](#). Journal of Plant Physiology. 2021, 263:153451. [10.1016/j.jplph.2021.153451](#)
99. Hu H, Wang H, Zhang Y, Kan B, Ding Y, Huang J: [Characterization of genes in guar gum biosynthesis based on quantitative RNA-sequencing in guar bean \(Cyamopsis tetragonoloba\)](#). Scientific Reports. 2019, 9:10991. [10.1038/s41598-019-47518-5](#)
100. Sharma S, Tyagi A, Srivastava H, et al.: [Exploring the edible gum \(galactomannan\) biosynthesis and its regulation during pod developmental stages in clusterbean using comparative transcriptomic approach](#). Scientific Reports. 2021, 11:4000. [10.1038/s41598-021-83507-3](#)
101. Verma S, Dhugga KS, Gill KS, Randhawa GS: [Downregulation of galactomannan galactosyltransferase in Cyamopsis tetragonoloba](#). Plant Cell, Tissue and Organ Culture (PCTOC). 2024, 159:29. [10.1007/s11240-024-02879-2](#)
102. Grant Reid JS, Edwards M, Gidley MJ, Clark AH: [Enzyme specificity in galactomannan biosynthesis](#). Planta. 1995, 195:489-495. [10.1007/BF00195705](#)
103. Francois LE, Donovan TJ, Maas EV: [Salinity effects on emergence, vegetative growth, and seed yield of guar](#). Agronomy Journal. 1990, 82:587-592. [10.2134/agronj1990.00021962008200030030x](#)
104. Sandhu APS, Randhawa GS, Dhugga KS: [Plant cell wall matrix polysaccharide biosynthesis](#). Molecular Plant. 2009, 2:840-850. [10.1093/mp/ssp056](#)
105. Edwards M, Bulpin PV, Dea IC, Reid JS: [Biosynthesis of legume-seed galactomannans in vitro](#). Planta. 1989, 178:41-51. [10.1007/BF00392525](#)
106. Dhugga KS, Barreiro R, Whitten B, et al.: [Guar seed \$\beta\$ -mannan synthase is a member of the cellulose synthase super gene family](#). Science. 2004, 303:363-366. [10.1126/science.1090908](#)

How to cite this article:

. P, Jangra S, Chaudhary V, et al. (May 20, 2026) Biotechnological Interventions in Unravelling the Genetic Treasure of an Orphan Crop, Cluster Bean (*Cyamopsis tetragonoloba* L.). Curues J. Agri. Food Sci 2 : es44497-025-00069-2. DOI <https://doi.org/10.7759/s44497-025-00069-2>

107. Goubet F, Barton CJ, Mortimer JC, et al.: [Cell wall glucomannan in Arabidopsis is synthesised by CSLA glycosyltransferases, and influences the progression of embryogenesis](#). The Plant Journal. 2009, 60:527-538. [10.1111/j.1365-313x.2009.03977.x](#)

How to cite this article:

. P, Jangra S, Chaudhary V, et al. (May 20, 2026) Biotechnological Interventions in Unravelling the Genetic Treasure of an Orphan Crop, Cluster Bean (*Cyamopsis tetragonoloba* L.). Curues J. Agri. Food Sci 2 : es44497-025-00069-2. DOI <https://doi.org/10.7759/s44497-025-00069-2>