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Research Article

Assessment of DAMD Molecular Markers in Genetic Diversity of Ricebean

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Abstract

Rice bean (*Vigna umbellata*) is an underutilized legume crop with significant nutritional and economic benefits. Despite this, rice bean remains underexploited due to limited information available on genetic resources. This study therefore focuses on assessing the genetic diversity of 20 rice bean genotypes collected from various geographic regions of India using Directed Amplification of Minisatellite DNA (DAMD) markers. Cluster analysis using Unweighted Pair Group Method with Arithmetic Mean (UPGMA) identified two broad clusters with subclades, cluster 1 included genotypes IC 524537, IC 524538, and IC 524542, while cluster 2 included genotypes IC 419603 and IC 524083 reflecting the genetic relationships among the genotypes. Highest eigen value of IC 526400, IC 419588 & IC 419602 were calculated by PCA that can support the development of desirable cultivars for future breeding programs.

Keywords: *DAMD, Ricebean, Genetic diversity.*

Introduction

Rice bean (*Vigna umbellate*_(Thunb) Ohwi and Ohashi), has great potential for complementing its nutritional and potential (Nayak, *et al*., 2022). *Ceratotropis, Plectotropis, and Vigna* are the only three subgenera of Vigna that have undergone domestication and cultivation (Pandiyan, *et al*., 2012). Rice bean is primarily grown in Nepal, Bhutan, and Northeast India extending to Myanmar, Southern China, Northern Thailand, Laos, Vietnam, Indonesia, and East Timor (Pattanayak, *et al*., 2019). In India, its cultivation is mainly limited to tribal areas of the Northeastern hills and the hilly regions of the Western and Eastern Ghats. In the Northeastern Hill Region of India, rice bean is predominantly grown under rainfed conditions within mixed farming systems, kitchen gardens and backyards (Das, *et al*., 2016). It is also known as climbing mountain bean (Saini and Chopra, 2012; Bhagyawant, *et al*., 2019), and used as a vegetable, pulse, fermented pulse, fodder, and in traditional medicine. Rice bean seeds contain about 60% digestible proteins, enriched with essential amino amino acids acid like methionine, tryptophan, lysine, tyrosine, and valine (Kaur, *et al*., 2013; Gupta, *et al*., 2018a). Moreover, plant seeds encompass bioactive peptides with many health promoting activity (Gupta, *et al*., 2024; Gupta, *et al*., 2024; Gupta, *et al*., 2022; Gupta, *et al*., 2021; Gupta, *et al*., 2019a; Gupta, *et al*., 2019b; Gupta, *et al*., 2018a; Gupta, *et al*., 2018b; Gupta, *et al*., 2018c; Gupta, *et al*., 2018d; Gupta, *et al*., 2016; Gupta, *et al*., 2017).

Studies on genetic variation in seed crops offer valuable insights for selecting parental material, thereby aiding in the development of breeding strategies that enhance yield, nutritional quality, and resistance to biotic and abiotic stresses (Raina *et al*., 2022). (Bhagyawant and Srivastava 2008a). However, studies of genetic diversity using many cultivated and wild genotypes from many Asian countries conducted in rice bean are pretty less, and the level and geographic cline of genetic diversity of rice bean is unknown (Tian *et al*., 2013)). To date, there are only a few reports on the intraspecies molecular diversity in *V. umbellata* analysed using RAPD (Gupta & Bhagyawant, 2021b), AFLP, ISSR, DAMD and SSR markers (Bajracharya, *et al*., 2008; Muthusamy, *et al*., 2008; Thakur, *et al*.,

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2017; Tian, *et al*., 2013; Iangrai, *et al*., 2017). Understanding the genetic variability among rice bean populations can lead to the development of superior cultivars with desirable traits (Gupta & Bhagyawant, 2021c).

PCR-based markers are popular due to their technical simplicity, ability to quickly screen large numbers of samples, and suitability for use in a moderately equipped laboratory (Bhagyawant, 2016). Currently, molecular markers are routinely used to verify plenty of agronomic traits (Zietkiewicz *et al*., 1994; Bhagyawant, 2016). They are primarily used for analyzing genetic diversity (Singh *et al*., 2014), DNA polymorphisms (Yadav *et al*., 2015), phylogeny (Yadav *et al*., 2016), gene tagging (Hakim *et al*., 2019) and quantitative trait loci (QTL) mapping (Obala *et al*., 2020).

Molecular markers, especially DNA-based markers, have revolutionized by providing detailed insights into the genetic makeup of crops (Hasan *et al*., 2021). Among the various molecular markers available, Directed Amplification of Minisatellite DNA (DAMD) markers have emerged as a powerful tool for genetic diversity studies. This study seeks to assess the genetic diversity of rice bean genotypes using DAMD, focusing on genotypes from varied geographic regions of India. The aim is to contribute valuable insights for future breeding programs involving rice bean.

Materials and Methods

Seed Material

Twenty rice bean (*Vigna umbellata*) genotypes were provided by the National Bureau of Plant Genetic Resources (NBPGR), India under a Material Transfer Agreement (MTA). Details of the rice bean genotypes are listed in Table 1.

Genomic DNA Extraction

Total genomic DNA from each accession's seeds was extracted using the method of Krishna and Jawali (1997) (Bhadkaria *et al*., 2020). The rice bean seeds were soaked overnight, then crushed in microcentrifuge tubes. Extraction buffer was added to these tubes, followed by incubation at 65°C for 10 minutes. Potassium acetate was then added, and the mixture was rested for 20 minutes at 4°C. The supernatant was combined with 75% isopropanol and 2.5 M ammonium acetate, while the pellet was rinsed with ethanol and re-suspended in elution buffer. The buffer was electrophoresed at 70 volts and 128 milliamperes for 90 minutes at 37°C using an electrophoretic unit. The quality of the extracted DNA was checked under a UV-transilluminator.

PCR Amplifications

PCR amplifications were carried out for 35 cycles with an initial denaturation at 95°C for 5 minutes. This was followed by denaturation for 1 minute, annealing at 55°C for 1 minute, and extension at 72°C for 4 minutes, with a final extension for 10 minutes in a Eppendorf Machine gradient thermal cycler. The 25 µL reaction mixture contained 20 ng template DNA (5 µL), 3.75 µL of 10X PCR buffer, 200 µM of each dNTP, 0.4 µM primer (2 µL), and 0.5 units of Taq DNA polymerase (3 µL), with the final volume made up by Milli-Q water. The primers used are listed in Table 2.

Agarose Gel Electrophoresis

The PCR products were analysed on a 1.5% agarose gel in 1X TAE buffer and electrophoresed at 60 V for 2 hours. Standard molecular weight DNA markers of 1 Kb from Fragments were used to assess the fractionation range of PCR products.

Statistics

The presence or absence of each band was indicated through symbols 1 and 0, respectively. A similarity matrix was generated using Euclidean's coefficient similarity. This similarity matrix was then clustered using the unweighted pair group method with arithmetic average (UPGMA) with PAST version 4.03 to determine the genetic relationships.

Results

Genetic Fingerprinting Analysis

Ricebean genotypes were screened using 05 DAMD marker, which targets tandemly repeated regions of a genome, is promising alternative marker approaches that have developed. It has been discovered that DAMD-PCR markers are more useful than ISSR markers for identifying intra- and interspecies genetic variation as well as linkages between genotypes (Amirmoradi *et al*., 2012).

The analysis demonstrated that DAMD 1 and DAMD 2 marker generated a polymorphic banding pattern across the twenty rice bean genotypes, depicted in Figure 2a & 2b. Specifically, no monomorphic bands were observed, indicating diversity in the amplified DNA regions among the genotypes tested. This suggests ample genetic variation in the loci targeted by these marker. The total number of bands amplified across the 20 genotypes was 90, with 2 marker, resulting in an average of 45 bands per primer and 2.25 bands per primer per genotype. The distribution of these bands was consistent across all samples, further emphasizing the polymorphic nature of the bands produced by the DAMD 1 marker. In contrast, the three additional marker tested did not amplify DNA in any of the rice bean genotypes, they are unable to locate complementary sequences in the genomic DNA (Gautam *et al*., 2016). The non-amplification of primers has also been documented in other crop plants (Bhagyawant and Srivastava 2008b). Among the 3' anchored marker, the primer with the sequence CTCTG amplified the fewest bands (50), while the primer GGCAG amplified the highest number of bands (70) across the 20 genotypes, as shown in Figures 2a and 2b. These results highlight the variability in primer efficacy and the importance of selecting suitable marker for genetic analysis.

Clustering Pattern and Similarity Index

The clustering pattern of the rice bean genotypes was assessed using bioinformatics software PAST version 4.03 to generate similarity indices based on pairwise comparisons. The UPGMA was employed to construct a dendrogram, illustrating the genetic relationships (Figure 3). The similarity index values ranged from 0.01 to 2.00, with an average value of 0.10, indicating varying degrees of genetic relatedness among the genotypes. The UPGMA cluster analysis (Yadav *et al*., 2022) revealed two broad clusters of rice bean genotypes; cluster 1 comprised genotypes IC 524537, IC 524538, and IC 524542 in one group, a subclade within this cluster included IC 524094, IC 524451, and IC 524464, another subclade contained IC 419518, IC 369282, IC 419517, IC 411641, IC 411730, and IC 416680, whereas, cluster 2 comprised genotypes IC 419603 and IC 524083 in one group, a subclade within this cluster included IC 419588, IC 419602, and IC 526400, another subclade contained IC 419590, IC 526614, and IC 526723.

The lowest pairwise genetic distance was observed between the two major clusters analysed using primer DAMD 1, suggesting a high degree of genetic similarity between these groups. The clustering analysis did not reveal a well-defined pattern among the genotypes, indicating limited genetic differentiation within the studied samples.

PAST software (version 4.03) was used to perform Principal Component Analysis (PCA) and create a 2D graphic from various marker across the genotypes. Figure 4 presents the hypothetical variables accounting for potential variance in the multivariate data. The PCA results revealed four main components, explaining 44.561%, 32.074%, 19% and 4.3% of the total genetic variation, with eigenvalues of 0.4632, 0.3334, 0.1979 and 0.0448, respectively. Highest eigen values were observed for IC 526400, IC 419588 & IC 419602.

Table 1: Rice bean accessions with their agronomic details

S. N.	Accession No.	Cultivated	Color	Biological Status	Latitude	Longitude
1	IC 369282	Jodhpur, Rajasthan	Pale brown	Landrace	26.2389° N	73.0243° E
2	IC 411641	Jodhpur, Rajasthan	Pale Brown	Landrace	26.2389° N	73.0243° E
3	IC 411730	Barmer, Rajasthan	Reddish Brown	Cultivated	25.7521° N	71.3967° E
4	IC 416680	Sirohi, Rajasthan	Brown	Cultivated	24.8852° N	72.8575° E
5	IC 419517	Barmer, Rajasthan	Pale Brown	Wild	25.7521° N	71.3967° E
6	IC 419518	Barmer, Rajasthan	Brown	Cultivated	25.7521° N	71.3967° E
7	IC 419588	Kutch, Gujarat	Reddish Brown	Cultivated	23.7337° N	69.8597° E
8	IC 419590	Arid Region Rajasthan	Brown	Cultivated	27.0238° N	74.2179° E
9	IC 419602	Kutch, Gujarat	Brown	Wild	23.7337° N	69.8597° E
10	IC 419603	Arid Region India	Reddish Brown	Cultivated	20.5937° N	78.9629° E
11	IC 524083	Kutch, Gujarat	Pale Brown	Cultivated	23.7337° N	69.8597° E
12	IC 524094	Arid Region India	Reddish Brown	Cultivated	20.5937° N	78.9629° E
13	IC 524451	Arid Region India	Pale Brown	Cultivated	20.5937° N	78.9629° E
14	IC 524464	Hisar, Haryana	Brown	Cultivated	29.1492° N	75.7217° E
15	IC 524537	Hisar, Haryana	Brown	Cultivated	29.1492° N	75.7217° E
16	IC 524538	Jaisalmer, Rajasthan	Brown	Wild	26.9157° N	70.9083° E
17	IC 524542	Churu, Rajasthan	Brown	Wild	28.2925° N	74.9707° E
18	IC 526400	Arid Region India	Brown	Cultivated	20.5937° N	78.9629° E
19	IC 526614	Arid Region India	Brown	Cultivated	20.5937° N	78.9629° E
20	IC 526723	Hisar, Haryana	Reddish Brown	Cultivated	29.1492° N	75.7217° E

Table 2: List of primers used for the amplifications of rice bean DNA

S.N.	Primer	Anchor Sequence (5′- 3′)	Length(bp)	Tm(°C)
1.	DAMD 1	GGC AGG ATT GAA GC	14	44.0
2.	DAMD 2	CTC TGG GTG TCG TGC	15	53.3
3.	DAMD 3	ACAGGGGTGGGG	12	42.0

4.	DAMD 5	AGGAGGAGGGGAAGG	15	53.3
5.	DAMD 6	GAGGGTGGCGGCTCT	15	56.0

Figures

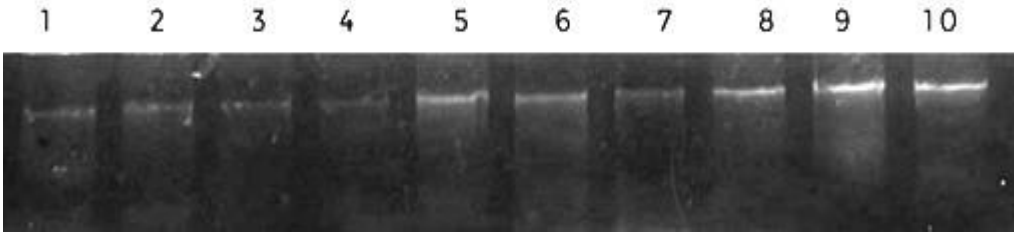

Fig 1: Genomic DNA concentration of rice bean accessions

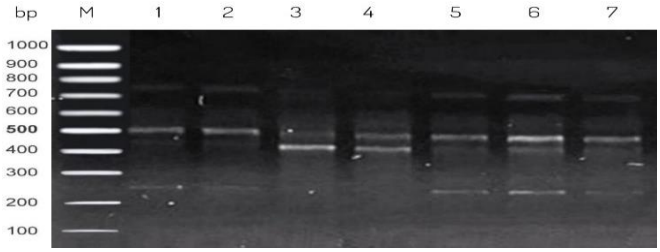

Fig 2a: PCR amplified products of seven accessions with DAMD 1

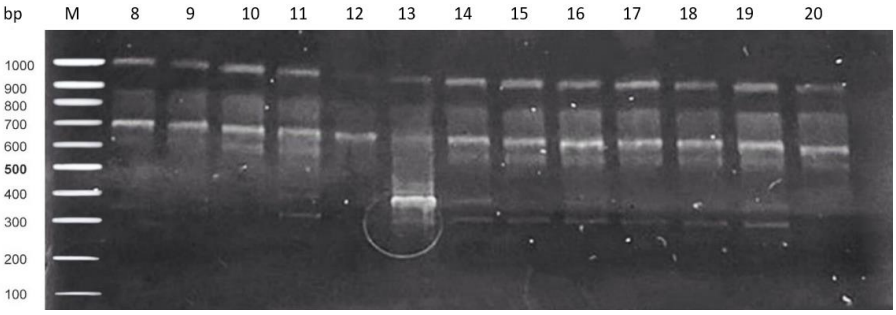

Fig 2b: PCR amplified products of thirteen accessions with DAMD 1

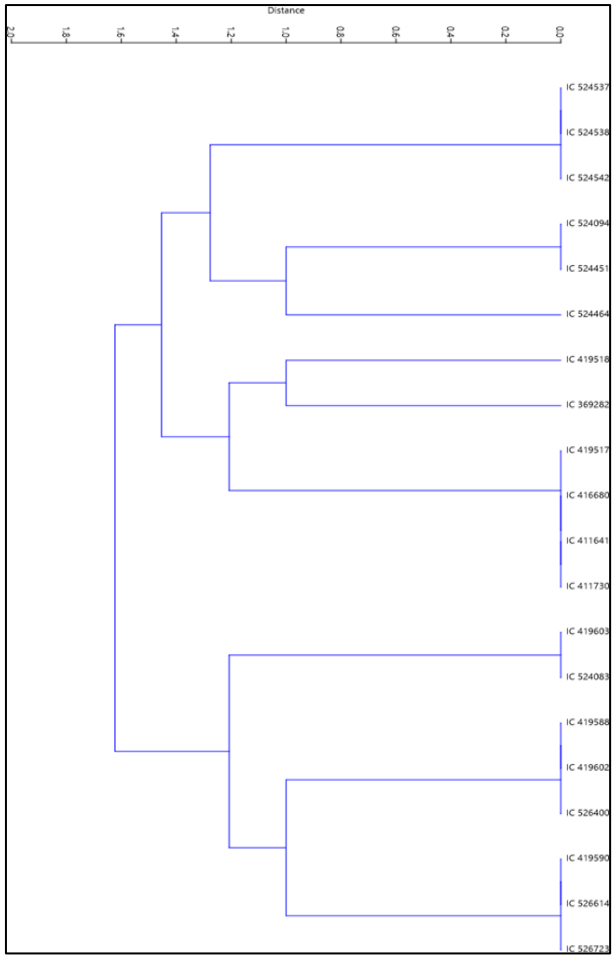

Fig 3: Dendrogram of rice bean accessions

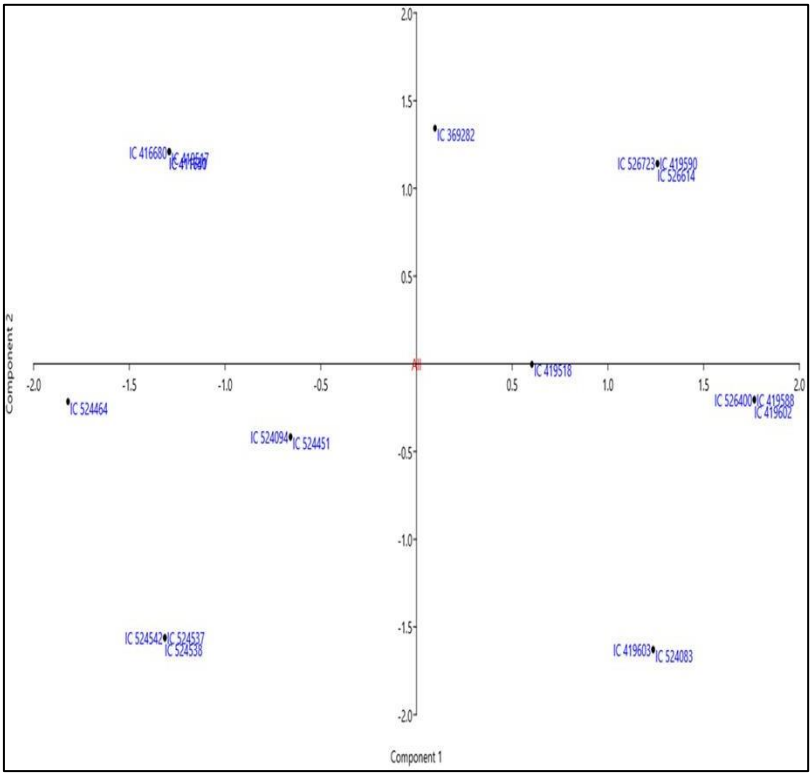

Fig 4: Principal component analysis (PCA) of different rice bean accessions

Discussion

The genetic fingerprinting results using DAMD marker revealed polymorphic pattern across rice bean genotypes. This uniformity suggests that the loci targeted by DAMD 1 are highly conserved among the genotypes studied, which could be attributed to a narrow genetic base within these samples.

The amplification of 90 bands across the genotypes, demonstrates the efficiency of DAMD marker in generating genetic fingerprints. However, the lack of polymorphism in the banding pattern limits the ability to distinguish between genotypes based on these markers (Abouseada *et al*., 2023). This finding underscores the need for incorporating additional genetic markers or techniques to provide a more comprehensive assessment of genetic diversity.

The variability in the number of bands amplified by different marker highlights the importance of primer selection in genetic studies (Amiteye, 2021). The primer GGCAG, which amplified the maximum number of bands i.e. 70, proved to be the most effective in this study, while the primer CTCTG, which amplified the fewest bands i.e. 50, was less effective. These differences emphasize the need for careful primer selection to maximize the detection of genetic variation. The lack of amplification by the three additional marker tested suggests that they were not suitable for the rice bean genotypes studied (Guan *et al*., 2022). This could be due to mismatches between the primer sequences and the target DNA regions or other factors affecting primer binding and amplification efficiency. Future studies should explore a broader range of marker and optimize PCR conditions to improve amplification success and resolution of genetic diversity (Gupta & Bhagyawant, 2021a).

The cluster analysis revealed two major clusters of rice bean genotypes with varying levels of similarity. These clusters provide a visual representation of the genetic relationships and can be used to identify closely related genotypes. The presence of subclades within the clusters indicates some level of genetic differentiation among the genotypes. This finding aligns with the polymorphic banding pattern observed in the genetic fingerprinting analysis, further indicating a narrow genetic base (Desalegne *et al*., 2016). Consequently, the current genotypes show low genetic polymorphism due to their genetic homogeneity and self-pollinating fertilization mode (Ajibade *et al*., 2000; Brink and Jansen 2006). This could be a result of selective breeding practices aimed at enhancing specific agronomic traits, such as yield potential and drought resistance.

Implications for Breeding Programs

The insights gained from this study have important implications for breeding programs aimed at improving rice bean genotypes. The limited genetic variation observed suggests that there may be a need to introduce new genetic material to broaden the genetic base and enhance the potential for breeding diverse and resilient rice bean varieties (Salgotra *et al*., 2023). This could involve the incorporation of genotypes from different geographic regions. The identification of closely related genotypes through cluster analysis can also inform breeding strategies by highlighting potential parental combinations for hybridization. By selecting genetically diverse parents, breeders can maximize the potential for generating progeny with desirable agronomic traits and increased genetic diversity (Swarup *et al*., 2021).

To gain a more comprehensive understanding of the genetic diversity in rice beans, future studies should incorporate a broader range of genetic markers, such as Simple Sequence Repeats (SSRs) or Single Nucleotide Polymorphisms (SNPs) (Bhagyawant *et al*., 2015). These markers can provide higher resolution, facilitating a more detailed assessment of genetic diversity and relationships. Additionally, increasing the sample size and including genotypes from diverse geographic regions can enhance the generalizability of the findings.

Conclusion

This study provides a foundational understanding of genetic diversity and relationships among twenty rice bean genotypes. The findings highlight the genetic variation observed using DAMD marker and need for incorporating additional marker techniques to capture more genetic diversity. The insights gained can be utilized in executing breeding programs. Future research on expanding the genetic tool kit and sample diversity of rice bean genetics is underway.

References

1. Abouseada, H. H, *et al*. "Genetic diversity analysis in wheat cultivars using SCoT and ISSR markers, chloroplast DNA barcoding and grain SEM." *BMC Plant Biology* 23.1 (2023): 193.
2. Ajibade, S. R., Weeden, N. F. and Chite, S. M. "Inter simple sequence repeat analysis of genetic relationships in the genus Vigna." *Euphytica* 111 (2000): 47-55.
3. Amirmoradi, B., Reza, T. and Ezaat, K. "Comparison of genetic variation and differentiation among annual Cicer species using start codon targeted (SCoT) polymorphism, DAMD-PCR, and ISSR markers." Plant systematics and evolution 298 (2012): 1679-1688.
4. Amiteye, S. "Basic concepts and methodologies of DNA marker systems in plant molecular breeding." *Heliyon* 7.10 (2021).
5. Bajracharya, J. "New Approaches in Varietal Research and Improvement at the National level: Quality Seed Demand and Supply Enhancing Access and Utilization for Food Security." *Fourth National Seed Seminar*. (2008): 19-20.
6. Bhadkaria, A, *et al*. "ISSR-PCR approach as a means of studying genetic variation in moth bean (Vigna aconitifolia (Jacq.) Maréchal)." *Biocatalysis and Agricultural Biotechnology* 30 (2020): 101827.
7. Sameer, S.B. "RAPD-SCAR markers: an interface tool for authentication of traits." *Journal of Biosciences and Medicines* 4.1 (2015): 1-9.
8. Sameer, S.B, *et al*. "Multivariate biochemical characterization of rice bean (Vigna umbellata) seeds for nutritional enhancement." *Biocatalysis and Agricultural Biotechnology* 20 (2019): 101193.
9. Sameer, S.B. and N. Srivastava. "Assessment of antinutritional factors and protein content in the seeds of chickpea cultivars and their divergence." *Journal of Cell and Tissue Research* 8.1 (2008a): 1333.
10. Sameer, S.B. and N. Srivastava. "Genetic fingerprinting of chickpea (*Cicer arietinum* L.) germplasm using ISSR markers and their relationships." *African Journal of Biotechnology* 7 (2008b): 24
11. Sameer, S.B., Neha, G., Ajay, G., Chaturvedi, S. K. and Nidhi, S. "Molecular diversity assessment in chickpea through RAPD and ISSR markers." *World Journal of Agricultural Research* 3.6 (2015): 192-197.
12. Das, G., Jayanta, K.P., Sameer, K. S., Sushanto, G. and Han-Seung, S. "Diversity of traditional and fermented foods of the Seven Sister states of India and their nutritional and nutraceutical potential: a review." *Frontiers in Life Science*, *9* (2016): 292-312.
13. Desalegne, A. B., Suheb, M., Kifle, D. and Michael, P. T. "Assessment of genetic diversity in Ethiopian cowpea [Vigna unguiculata (L.) Walp.] germplasm using simple sequence repeat markers." *Plant Molecular Biology Reporter* 34 (2016): 978-992.
14. Gautam, A. K., N. Gupta., R. Bhadkariya., N. Srivastava. and Sameer, S.B.. "Genetic diversity analysis in chickpea employing ISSR markers." *Agrotechnology* 5.2 (2016): 2168-9881.
15. Guan, J, *et al*. "Genomic analyses of rice bean landraces reveal adaptation and yield related loci to accelerate breeding." *Nature Communications* 13.1 (2022): 5707.
16. Gupta, N., Nidhi, S., Pramod, K.S. and Sameer, S. B. "Phytochemical evaluation of moth bean (*Vigna aconitifolia* L.) seeds and their divergence." *Biochemistry Research International*, *2016* (2016): 1–6.
17. Gupta, N., N. Shrivastava. & Sameer, S.B. "Multivariate analysis based on nutritional value, antinutritional profile and antioxidant capacity of forty chickpea genotypes

grown in India." *Journal of Nutrition & Food Sciences*, *7* (2017): 3.
18. Gupta, N. & Sameer, S.B. "Angiotensin-I converting enzyme (ACE-I) inhibitory and antiproliferative potential of chickpea seed protein hydrolysate. *Annals of Plant Sciences*, 7 (2018a): 2149–2153.
19. Gupta, N., Prakash, S. B., and Sameer, S. B. "Chickpea lectin inhibits human breast cancer cell proliferation and induces apoptosis through cell cycle arrest." *Protein and Peptide Letters*, *25* (2018b): 492–499.
20. Gupta, N., Kumar, G. and Sameer, S.B. "Biochemical characterisation of lectin from wild chickpea (*Cicer reticulatum* L.) with potential inhibitory action against human cancer cells." *Journal of Food Biochemistry*, *43* (2018c): e12712.
21. Gupta, N., Nidhi, S. and Sameer, S. B. "Vicilin—A major storage protein of mungbean exhibits antioxidative potential, antiproliferative effects and ACE inhibitory activity." *PLOS ONE*, *13* (2018d): e0191265.
22. Gupta, N. & Sameer, S.B. "Enzymatic treatment improves ACE-I inhibition and antiproliferative potential of chickpea." *Vegetos*, *32* (2019a): 363–369.
23. Gupta, N. & Sameer, S.B. "Impact of hydrolysis on functional properties, antioxidant, ACE-I inhibitory and antiproliferative activity of *Cicer arietinum* and *Cicer reticulatum* hydrolysates." *Nutrire*, *44* (2019b): 5.
24. Gupta, N. & Sameer, S.B. "Analysis of Genetic Diversity in Chickpea Employing ISSR Markers." (2021a): 73-78.
25. Gupta, N. and Sameer, S.B. "RAPD analysis in kabuli chickpea seed accessions." (2021b): 6-9.
26. Gupta, N. & Sameer, S.B. "Testing DNA variation in Kabuli chickpea seeds through ISSR-PCR." (2021c): 1-4.
27. Gupta, N. & Sameer, S.B. "Bioactive peptide of *Cicer arietinum* L. induces apoptosis in human endometrial cancer via DNA fragmentation and cell cycle arrest." *3 Biotech*, *11*(2) (2021): 63.
28. Gupta, N., Sameer, Q., Saurabh, K.J., Mohammad, K.S., Kanika, V., Swapnil, S., Rizwan, H.K. and Sameer, S.B. "Chickpea peptide: A nutraceutical molecule corroborating neurodegenerative and ACE-I inhibition." *Nutrients*, *14* (2022): 4824.
29. Gupta, N., Sameer, S.B., Abeer, H. and Elsayed, F.A.A. "Exploring bioactivities from distinct Cicer protein hydrolysate as influenced by enzymatic hydrolysis." *CyTA-Journal of Food* 22.1 (2024): 2362688.
30. Gupta, N. "Mungbean seed protein: A potent functional food with health promoting biological potential." *International Journal of Advanced Biochemistry Research* 8(3); (2024): 659-664
31. Hakim, S, *et al*. "Illumina sequencing of 16S rRNA tag shows disparity in rhizobial and non-rhizobial diversity associated with root nodules of mung bean (Vigna radiata L.) growing in different habitats in Pakistan." *Microbiological research* 231 (2020): 126356.
32. Hasan, N., Sana, C., Neha, N., Nidhi, S. and Rafiul, A.L. "Recent advancements in molecular marker-assisted selection and applications in plant breeding programmes." *Journal of Genetic Engineering and Biotechnology* 19.1 (2021): 128.
33. Iangrai, B, *et al*. "Development and characterization of a new set of genomic microsatellite markers in rice bean (Vigna umbellata (Thunb.) Ohwi and Ohashi) and their utilization in genetic diversity analysis of collections from North East India." *PloS one* 12.7 (2017): e0179801.
34. Kaur, A, *et al*. "Grains, starch and protein characteristics of rice bean (Vigna umbellata) grown in Indian Himalaya regions." *Food Research International* 54.1 (2013): 102-110.
35. Krishna, T. G. and N. Jawali. "DNA isolation from single or half seeds suitable for random amplified polymorphic DNA analyses." *Analytical Biochemistry* 250.1 (1997): 125-127.
36. Muthusamy, S., Selvaraju, K. and Shanmugasundaram, P. "Efficiency of RAPD and ISSR markers system in accessing genetic variation of rice bean (Vigna umbellata) landraces." *Electronic Journal of Biotechnology* 11.3 (2008): 32-41
37. Nayak, H. S, *et al*. "Rice yield gaps and ni-

trogen-use efficiency in the Northwestern Indo-Gangetic Plains of India: Evidence based insights from heterogeneous farmers' practices." *Field Crops Research* 275 (2022): 108328.
38. Obala, J, *et al*. "Seed protein content and its relationships with agronomic traits in pigeonpea is controlled by both main and epistatic effects QTLs." *Scientific Reports* 10.1 (2020): 214.
39. Pandiyan, M, *et al*. "Diversity analysis of Vigna sp through morphological markers." *Wudpecker J Agric Res* 1.8 (2012): 335-340.
40. Pattanayak, A, *et al*. "Rice bean: a lesser known pulse with well-recognized potential." *Planta* 250 (2019): 873-890.
41. Raina, A., Rafiul, A.L., Mohammad, R.W. and Samiullah, K. "Plant breeding strategies for abiotic stress tolerance in cereals." In *Omics approach to manage abiotic stress in cereals*. Singapore: Springer Nature Singapore, (2022): 151-177.
42. Singh, P. K., Himanshu, S., Nidhi, S. and Sameer, S.B. "Analysis of genetic diversity among wild and cultivated chickpea genotypes employing ISSR and RAPD markers." *American Journal of Plant Sciences* 2014 (2014).
43. Saini, R. and Aparna, R.C. "In-vitro plant regeneration via somatic embryogenesis in rice-bean Vigna umbellata (Thunb.) Ohwi and Ohashi: an underutilized and recalcitrant grain legume." (2012): 452-457.
44. Salgotra, R. K. and Bhagirath, S.C. "Genetic diversity, conservation, and utilization of plant genetic resources." *Genes* 14.1 (2023): 174.
45. Swarup, S, *et al*. "Genetic diversity is indispensable for plant breeding to improve crops." *Crop Science* 61.2 (2021): 839-852.
46. Thakur, S., N. Bhardwaj. and Chahota, R. K. "Evaluation of genetic diversity in ricebean [Vigna umbellata (Thunb.) Ohwi and Ohashi] germplasm using SSR markers." *Electronic Journal of Plant Breeding* 8.2 (2017): 674-679.
47. Tian, J., T. Isemura., A. Kaga., Vaughan, D. A. and N. Tomooka. "Genetic diversity of the rice bean (Vigna umbellata) genepool as assessed by SSR markers." *Genome* 56, 12 (2013): 717-727.
48. Yadav, P., Koul, K. K., N. Shrivastava., Mendaki, M. J. and Sameer, S.B. "DNA polymorphisms in chickpea accessions as revealed by PCR-based markers." *Cellular and Molecular Biology* 61.5 (2015): 84-90.
49. Yadav, P., Koul, K. K., N. Shrivastava., Mendaki, M. J. and Sameer, S.B. "ITS-PCR sequencing approach deciphers molecular phylogeny in chickpea." *Plant Biosystems-An International Journal Dealing with all Aspects of Plant Biology* 151.3 (2017): 429-435.
50. Yadav, P., Yadav, K. K. and Sameer, S.B. "Molecular assessment of wild and cultivated Cicer species using ISSR markers." *Ecological Genetics and Genomics* 25 (2022): 100137.
51. Zietkiewicz, E., Antoni, R. and Damian, L. "Genome fingerprinting by simple sequence repeat (SSR)-anchored polymerase chain reaction amplification." *Genomics* 20.2 (1994): 176-183.

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