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# 12. Genetic Diversity Assessment in Rice Bean (*Vigna umbellate*) (Thumb.) Ohwi & Ohashi of Northeast, India

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***Abstract:***

*Introduction-Rice bean (Vigna umbellata), is a warm seasonal annual legume grown in Northeast Asia. It is an important crop for shifting cultivation or kitchen garden which is consumed as vegetables and pulses.*

*Objective-This study is attempted to assess genomic diversity of this legume crop using SSR (Simple sequence repeat) markers.*

*Methodology- Genetic variation between five land races of rice bean were evaluated using SSR markers. SSR primers generated 80 amplification products out of which 26 were polymorphic. Mean PIC (polymorphism information content) for SSR marker (0.34) suggested that the marker was effective in determining polymorphism. The dendrogram constructed using SSR marker were highly correlated. Similarity indices ranged from 0.15 to 0.45.*

*Conclusion- SSR marker was found to be useful for the genetic diversity studies in Vigna umbellata and identify variation within landraces*

***Keywords:***

*Vigna umbellate, Rice bean, Genetic diversity, SSR marker, Northeast India.*

## 12.1 Introduction:

Vigna umbellate (Thumb.) Ohwi and Ohashi commonly known as rice bean is cultivated mainly in Nepal, Bhutan, Northeast India which extends through Myanmar, Southern China, Thailand, Laos and Vietnam to Indonesia (Tomooka *et al*., 2002; Tomooka *et al*., 2003). It is an important underutilized leguminous plant used as vegetable, traditional medicine and fodder in Southeast Asian countries (Wu *et al*., 2001).

Most research conducted on rice bean has focused on its high level of resistance to the major storage pest bruchid beetles (*Callosobruchus* sp.) (Tomooka *et al*., 2000; Kashiwaba *et al*., 2003; Somta *et al*., 2006). This crop is free from diseases such as yellow mosaic virus, Cercospora and bacterial leaf spot (Arora, 1980; Pandiyan, 2008).

Classification of various subgenera, species and subspecies is based primarily on morphological attributes but due to different environmental factors morphological characters may vary from place to place.

Limited studies with genetic diversity have been carried using limited number of cultivated and wild rice bean accessions from Asian countries (Seehalak *et al*, 2006; Muthusamy *et al*., 2008; Bajracharya *et al*., 2010).

Rice bean is considered as underexploited potential legume, this study was undertaken to assess the genomic diversity among widely distributed variety of rice bean using SSR primer and determine their relationships.

**12.2 Material and Methods:**

Total five *Vigna umbellate* accessions were procured from Sikkim, Nagaland, Manipur, Mizoram and Arunachal Pradesh during January- February, 2019 and used in this experiment (Table 12.1).

All the plants (single plant per accession) were sown in plastic pots in Botanical Garden of Department of Botany, Sikkim University and maintained as per the standard package and practices.

Temperature of the green house was maintained above 20 $^{o}$C. The number of pod-bearing lateral branches and days to first flower opening were recorded.

**Table 12.1- Five accessions of *Vigna umbellata* used for SSR analysis**

| Place | Accession number |
|---|---|
| Sikkim | VU-1 |
| Nagaland | VU-2 |
| Manipur | VU-3 |
| Mizoram | VU-4 |
| Arunachal Pradesh | VU-5 |

**DNA extraction:**

Total DNA was isolated from 150 to 350 mg of young leaves of a single plant per accession by freezing in liquid nitrogen. Genomic DNA was extracted and purified according to McCouch *et al*., (1988).

**Polymerase Chain reaction (PCR) amplification:**

Fourteen primers developed for rice bean were used to assess the genetic diversity and the information on these primers is shown (Table) DNA amplification reactions were performed in a volume of 25µL containing 10mM Tris-HCl (pH 8.3), 50 mM KCl, 2 mM $MgCl_2$, 50 mM each of dATP, dCTP, dTTP, 10ng of a single random primer, 25ng of genomic DNA and 2 units of *Ampli Taq* DNA polymerase (Perkin Elmer Cetus), topped with 50µL of sterilized mineral oil. Amplifications were performed in a thermal cycler (Perkin Elmer Cetus) programmed for 45 cycles for 1 min at 94°C, 1 min at 37°C and 2 min at 72°C.

**Gel electrophoresis:**

PCR amplified products of the amplicons were subject to gel electrophoresis using 1.5% agarose gel in 1X TBE buffer at 80V for 1 hour. 1kb DNA ladder (GIBCO BRL life technologies, Paisley, UK) as size marker and ethidium bromide was added to make the DNA bands visible and later observed under transilluminator.

**Data analysis:**

Genotyping data obtained from each of the marker system was used for assessing its discriminatory power by evaluating polymorphism information content (PIC). The PIC value and mean genetic diversity for each primer/ enzyme combination was calculated as part of summary statistics using Power Marker version 3.25 (Liu & Muse 2005) and was averaged over the fragments for each primer combination.

Each SSR marker was treated as a unit character and scored as a binary code (1/0). Data were scored for computer analysis on the basis of presence or absence of the amplified products.

If a product was present in a genotype, it was designated as "1", if absent it was designated as "0". Pair wise comparisons of genotypes, based on the presence or absence of unique or shared polymorphic products, were used to generate similarity coefficients (Jaccard, 1908).

The similarity coefficients were used to construct a dendrogram by UPGMA (un-weighted pair-group method with arithmetical average) (Sokal and Michener, 1958). The reliability and robustness of the dendrograms were tested by bootstrap analysis of 1000 iterations using the Winboot software (Yap & Nelson, 1996).

**12.3 Results:**

PCR amplification and SSR analysis was done for 5 rice bean cultivars. Altogether 14 SSR primers were used listed in Table 12.2. Out of which 8 primers were amplified and remaining 6 were not amplified. Amplified images were obtained after PCR amplification were scored in the excel sheet using binomial scoring i.e., 0 for the absence of band and 1 for the presence of band.

**Table 12.2- SSR primers used to detect polymorphism, number of bands for polymorphism per primer**

| Primer Sequence | | | | |
|---|---|---|---|---|
| **Primer** | **Forward** | **Reverse** | **Product Size Annealing (bp)** | **Temp (°C)** |
| MPU 15 | GAAGAGGAGGTTCAGAACAGT | CTTCTCCTCCAAGTCTTGTCA | 300 | 54 |
| MPU 27 | TTCTCACCTGTGTTGCTTATC | GCAGGCATATGATAGTGTGAT | 350 | 50 |
| MPU 85 | CCTCTTCCTCTTCTATCAACC | ATGTTTAATTACAACCCCACA | 550 | 60 |
| MPU_105 | CAAAGCTAGCATTAGAAGCAG | AAAAATACGTAAAAGGGATGG | 500 | 54 |
| MPU_204 | AAGCTTGTCACTGTCAAAAAG | ATGCAACACATGTCAACACT | 480 | 52 |
| MPU_234 | TAATAAGCTTGATCGTGATGC | AGCATTCATTGTAAGCAGAAG | 750 | 55 |
| MPU_434 | AATGGATCCCTTTTCTCTATG | TATTGGAATAGATCCCCTTGT | 800 | 60 |
| MPU_598 | ACACCCAGTATCTTCCTCTTC | TTGTGCTTTTGATTCTTTAGG | 520 | 54 |
| MPU 671 | AGCTTTCAATACAATCCATGA | AATGTGAGTGATGGTTGAGAC | 420 | 51 |
| MPU 698 | CCTTCCCTTTAGATGTGAAAT | ACATTGATAGCAGTGGAGAAA | 500 | 54 |
| MPU 700 | CCTTCCCTTTAGATGTGAAAT | ACATTGATAGCAGTGGAGAAA | 380 | 52 |
| MPU_777 | ATTCCTCCAAAACACAGTACC | GTGGGATAGGAGAAGCAT | 720 | 54 |
| MPU_783 | AGTTAAAACTCACCCACCCTA | TGGAGGTGAGTGAATAGATGA | 800 | 50 |
| MPU_817 | TGTTCTGCTGTAGTCCTCAAT | CAAATTTGCAGAAAAGGTATG | 590 | 54 |

Altogether 80 SSR markers were scored. The number of scorable markers produced per primer ranged from 4 to 5 and size of the products ranged from 300bp to 1000bp. (Figure 12.1-12.8). The similarity indices based on Jaccard's similarity coefficient varied from 0.15 to 0.45 with an average of 0.25055. VU3 showed the highest similarity index (0.45) and VU4 showed the lowest index (0.15).

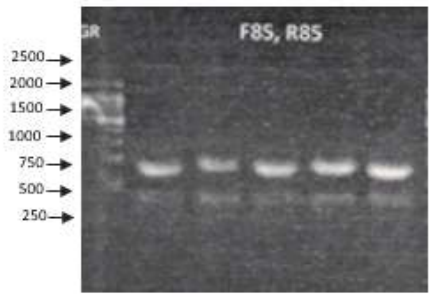


**Figure 12.1: SSR marker profile using F85, R85 primer for 5 land races of *Vigna umbellata***

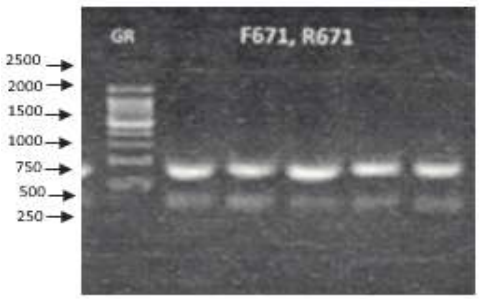


**Figure 12.2: SSR marker profile using F671, R671 primer for 5 land races of *Vigna umbellata***

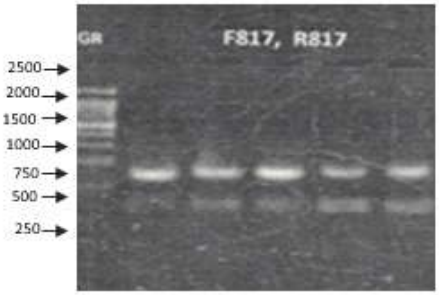


**Figure 12.3: SSR marker profile using F817, R817 primer for 5 land races of Vigna umbellate**

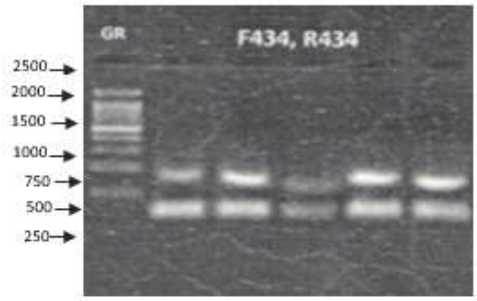


**Figure 12.4: SSR marker profile using F434, R434 primer for 5 land races of Vigna umbellata**

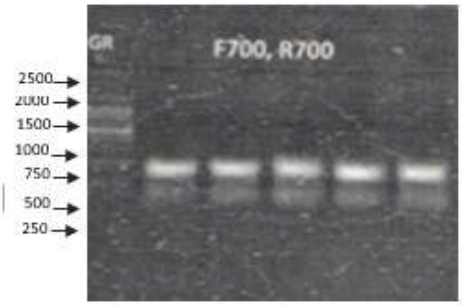


**Figure 12.5: SSR marker profile using F700, R700 primer for 5 land races of *Vigna umbellata***

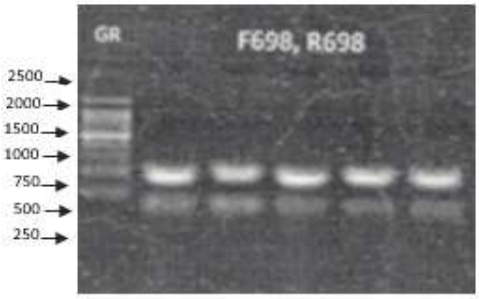


**Figure 12.6: SSR marker profile using F698, R698 primer for 5 land races of *Vigna umbellate***

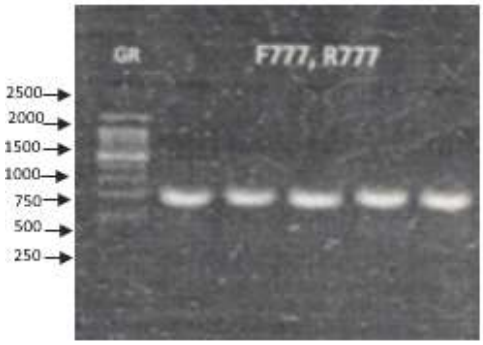


**Figure 12.7: SSR marker profile using F777, R777 primer for 5 land races of *Vigna umbellata***

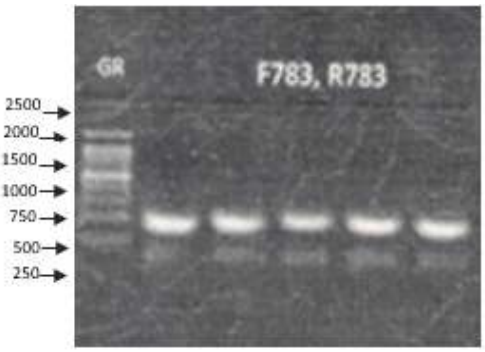


**Figure 12.8: SSR marker profile using F783, R783 primer for 5 land races of *Vigna umbellata***

**Table 12.3- Data on Jaccard's Similarity coefficient for 5 Rice bean cultivars for SSR**

| Genotype | VU1 | VU2 | VU3 | VU4 | VU5 |
|---|---|---|---|---|---|
| VU1 | | | | | |
| VU2 | 0.25 | | | | |
| VU3 | 0.45 | 0.25 | | | |
| VU4 | 0.15 | 0.17 | 0.15 | | |
| VU5 | 0.15 | 0.27 | 0.25 | 0.4 | |
| | 0.25 | | | | |
| | 0.17 | | | | |
| | 0.27 | | | | |
| | 0.15385 | | | | |
| | 0.25 | | | | |
| | 0.4 | | | | |
| **Average** | 0.25055 | | | | |
| **Maximum** | 0.45455 | | | | |
| **Minimum** | 0.15385 | | | | |
| | | | | | |

Out of 80 SSR markers generated, the total number of polymorphic markers were 26 and the percentage of polymorphism was found to be 33%. For the analyzed 80 SSR markers, the genetic values varied from 0.32 to 0.48 with an average at 0.442 and the polymorphic information content (PIC) content ranged from 0.27 (F85, R85) to 0.38 (F817, R817) with an average of 0.34.

**Table 12.4: Results of marker attributes for 8 amplified markers using SSR**

| Primer | Genetic Diversity (H) | PIC |
|---|---|---|
| F783, R783 | 0.48 | 0.36 |
| F434, R434 | 0.48 | 0.35 |

| Primer | Genetic Diversity (H) | PIC |
|---|---|---|
| F700, R700 | 0.48 | 0.34 |
| F698, R698 | 0.42 | 0.33 |
| F777, R777 | 0.44 | 0.34 |
| F671, R671 | 0.45 | 0.35 |
| F817, R817 | 0.47 | 0.38 |
| F85, R85 | 0.32 | 0.27 |

The UPGMA clustering based on Jaccard's similarity coefficient shown in Fig. 12.9 grouped 5 rice bean cultivars into two major clusters with good confidence limits arrived on bootstrapping at a replication of 1000. First cluster was divided into two subclusters, one with VU-2 and the other with VU-1 and VU-3 and the second cluster with VU-4 and VU-5. Hundred percent similarity was found between the two clusters.

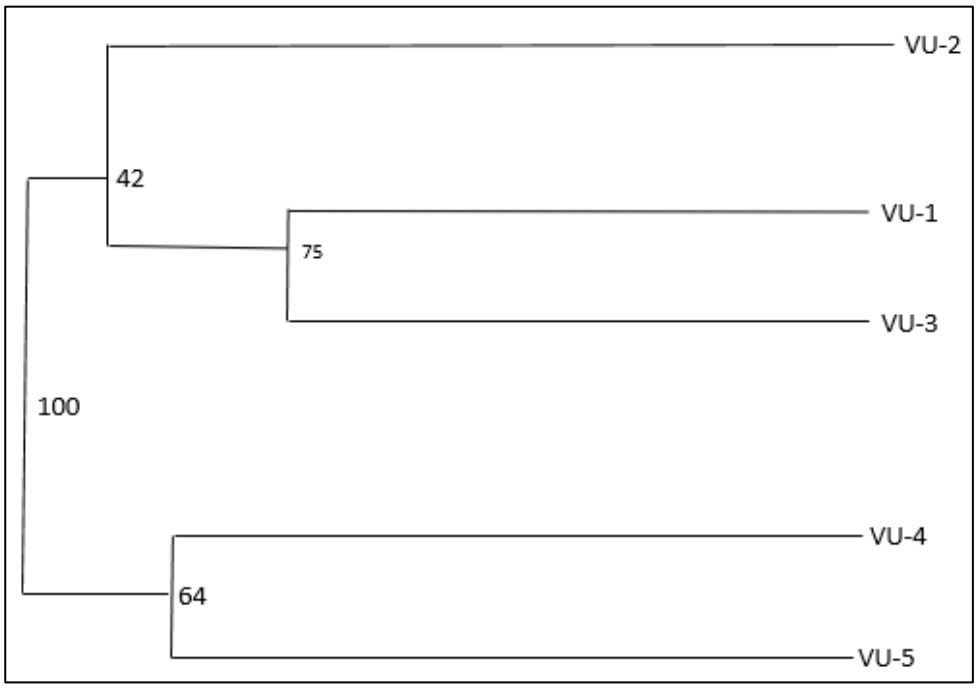


**Figure 12.9: UPGMA clustering obtained for 5 Rice bean cultivars genotypes based on Jaccard's similarity coefficients**

**12.4 Discussion:**

Rice bean, Vigna umbellate (Thumb.) Ohwi & Ohashi, a native of Southeast Asia (Burkill, 1935), is grown in India chiefly by the natives of the Eastern and Northeastern region (Watt, 1971). There is enormous variability in this tribal pulse. Through explorations conducted from 1970 onwards, about 300 collections have been made from the tribal-dominated mountainous tracts of Eastern (Bihar, Orissa) and Northeastern India (Assam, Meghalaya,

Manipur, Mizoram), where this crop is grown in fields under shifting cultivation with other crops like millets or as a kitchen garden crop in courtyards (Chandel *et al*., 1988). Rice bean is adopted to sub-humid regions and in general yields between 200 and 300 kg ha-1. It grows on a wide range of soil types including acid soils and is largely resistant to pests and disease. It is drought tolerant and will also tolerate some degree of water logging. The large range of diversity available in mountainous countries suggests that there is excellent potential for breeding to improve crop for a number of the traits desired by the farmers, so as to promote its use more widely.

The classification by farmers in these countries in terms of preferred traits (time to maturity, grain size and colour, growth habit) should be particularly useful in identifying genotypes for use as parents in breeding programme (Gautam *et al*., 2007)

Most of the protein requirements of the rural Indian population are met by legumes which constitute an important source of protein. However, the scanty information available on nutritional and functional components of Rice bean varieties grown in Northeastern India calls for the gap filling research to explore full potential of the bean.

With advent of genomic tools such as molecular markers, genetic maps etc., the genetic improvement of underutilized crops has been facilitated greatly and improved genotypes with enhanced trait values have been developed in several crop species (Sharma *et al*., 2013). Diversity estimates in crop plants provide a rationale for conservation strategies and support the selection of starting material for breeding programs (Laurentin & Karlovsky, 2007). Conventionally these are obtained by measuring polymorphism at morphological or DNA levels. The objective of this study was to assess genomic diversity in rice bean germplasm to elucidate their relationship and suggest parents for future breeding programs with a good yield.

Among the different rice bean cultivars, genetic variation was detected using SSR. Polymorphism was scored and used for UPGMA analysis to construct dendrograms. In the present study, the application of agarose gels gave a satisfactory resolution of PCR amplification products. The use of 1.5% agarose gel could resolve DNA products. Amplified products with molecular weights of 300-1000 base pair.

UPGMA clustering for the SSR data set NTSYS software (2.02) was used for the cluster analysis and phylogenetic tree construction. The UPGMA clustering based on Jaccard's similarity coefficients for SSR analysis grouped 5 rice bean cultivars into two major clusters with good confidence limits arrived on bootstrapping at a replication of 1000. First cluster was divided into two sub-clusters, one with VU-2 and the other with VU-1 and VU-3 and the second cluster with VU-4 and VU-5. Hundred percent similarity was found between the two clusters. Relatively low level of divergence observed between Rice bean accessions in the present study are similar to studies on Azuki bean reported based on nucleotide sequences (Fatokun *et al*., 1993; Kaga *et al*., 2000), seed proteins (Paino *et al*., 1990) and isozymes (Jaaska & Jaaska, 1990).

This study indicated that the use of SSR techniques to detect genetic variation at the level of DNA among rice bean was useful.

## 12.5 Conclusion:

Rice bean is a beneficial legume in the Himalayan region of India mostly in Northeastern states of India including Sikkim and adjoining Darjeeling hills of West Bengal. Genomic diversity of rice bean accessions presented in this study provides preliminary input for genetic studies.

Confirmation of mass multiplication protocol in elite genetic stock will be highly useful in multiplying this accession for breeding programs. However, limited number of accessions and less robust genotyping method used here restricts the relevance of the analysis for larger scientific inferences. Therefore, testing large number of primers from an extended collection using high-throughput genotyping is recommended for ascertaining genetic diversity scenario in this crop. Besides, lowering sequencing costs offer transcriptome analysis and genotyping by sequencing as some of the potential options for the development of species-specific markers- the success of which might open up newer vistas for molecular breeding in underutilized legumes in general and Rice bean in particular.

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