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A comparative analysis of genetic diversity across certain Mungbean and Urdbean cultivars of West Bengal, using ISSR markers

Swati Das (Sur), Surya Shekhar Das and Parthadeb Ghosh*

Cytogenetics and Plant Biotechnology Research Unit, Department of Botany, University of Kalyani, Kalyani, West Bengal, India

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ABSTRACT

The objective of the present study was to prepare a comparative account of the extent of genetic diversity and genetic relationships among the 9 Vigna varieties based on DNA data. A total number of 10 ISSR primers that produced polymorphic and reproducible fragments were selected to amplify genomic DNA of the genotypes under investigation. Ten primers amplified a total number of 624 bands under 104 loci across nine genotypes with average of 10.4 loci / primer. The cultivars exhibited an overall 76.92% polymorphism. The value of Jacaard's similarity coefficient ranged from 0.566 to 0.793. Panna showed the highest genetic diversity among all the five cultivars of mung bean, but was surprisingly and interestingly lower than any of the four cultivars of urd bean. The urd bean variety Kalindi seems to be genetically the closest one to the mung bean varieties studied. In comparison to other cultivars, Gautam showed the most genetic variability, suggesting utilization of this genotype over others for breeding programme and in transferring important characters into different cultivars. Overall, a high degree of genetic diversity among the 9 cultivars was recorded, which can be used for crop improvement especially keeping in mind the fact that mung bean has been reported to cross with a number of Vigna species when it is used as a female parent. The information generated from this study will be helpful to identify the germplasms, know their phylogenetic relationships and above all to construct an interspecific hybridization strategy.

Key words: Genetic diversity; *Vigna*; ISSR; Polymorphism; Crop improvement.

INTRODUCTION

Pulses in India have long been considered as the poor man's only source of protein. India is the largest importer, producer and consumer of pulses [1]. India accounts for 33% of the world area and 22% of the world production of pulses [2]. Pulses are not only a low cost source of protein for majority of Indian consumers but also a low cost substitute for vegetables in periods of high prices of vegetables. An improvement in pulses production can reduce the market prices, and create scope for further increase in demand for pulse crops by replacing some portion of the disproportionately high level of cereals in the consumption basket for a balanced diet. There is also scope for increasing the use of pulses in value added products such as papad, snack food, in the confectionery industry etc.

The genus *Vigna* contains several pulse species that are of considerable economic importance in many developing countries. Mung beans [*V. radiata* (L.) Wilczek] and urd beans [*V. mungo* (L.) Hepper] are important legume crops in India and are key dietary staples for millions of people. The crops are utilized in several ways, where seeds, sprouts and young pods are consumed as sources of protein, amino acids, vitamins and minerals, and plant parts are used as fodder and green manure. Mungbean protein is easily digested without flatulence. Both legumes adapt well to various cropping systems owing to their ability to fix atmospheric nitrogen (N_2) in symbiosis with the soil bacteria *Rhizobium* spp., rapid growth, and early maturity. They can be grown successfully in extreme environments (e.g., high temperatures, low rain fall, and poor soils) with few economic inputs. In addition to being an important pulse-yielding crop, mung bean assumes additional importance due to the fact that it has been reported to cross with a number of *Vigna* species when it is used as a female parent [3].

However, the global productivity of pulses is very low as compared to cereals. During the last five decades, annual pulse production in India has increased only marginally (from 12.70 million tones in 1960–1961 to 14.6 million tones in 2008–2009). Due to stagnant production, the net availability of pulses has come down from 60 gm/day/person in 1951 to 31 gm/day/person (Indian Council of Medical Research recommends 65 gm/day/capita) in 2008 [1].

Many biotic and abiotic stresses such as disease, insects, drought, high temperature, salinity and heavy metals limit mung bean yields. Despite the efforts of plant breeders during the past few decades, the development of sustainable resistance with higher yields has not been successful due to the narrow genetic diversity in the gene pool of the present cultivars [4]. It is imperative to further explore the genetic diversity available in this crop for better utilization of genetic resources in yield improvement. Genetic variability and divergence present in the materials is an important tool for any breeding programme.

Considering their socioeconomic importance, the crops are neglected in breeding research, particularly in the filed of marker assisted breeding. Molecular markers, unlike morphological markers, are not prone to environmental influences and have been found to be very useful to quantify accurately the extent of interspecific genetic diversity [5] and portray genetic relationships between plant groups [6]. DNA markers provide an opportunity to characterize genotypes and to measure genetic relationships more precisely than other markers [7]. Among the several DNA marker systems that are now being used in diversity studies of plants, inter simple sequence repeat (ISSR) [8] is an important one. It is a simple, rapid, reliable and inexpensive technique. Moreover, it is a better tool than RAPD for phylogenetic studies as the ISSR primers produce more information in terms of total number of loci and polymorphic bands [9]. ISSRs fingerprinting has been commonly used to identify germplasms, resolve uncertain parentage, and study genetic diversity, population genetics, taxonomy and phylogeny of many plant species [9].

Considering the potentials of the DNA marker based genetic diversity analysis for evolving systematic breeding strategies, the present study aimed to assess and analyze the nature and the extent of genetic diversity among the selected cultivars of mung bean and urd bean from West Bengal using inter simple sequence repeat (ISSR) marker. The authors previously studied the intraspecific genetic diversity of certain mung bean and urd bean cultivars separately [10, 11]. The present study compiles all the cultivars to reveal the interspecific genetic diversity of the two species of *Vigna*. The outcome will be helpful in preparing a comparative account of genetic diversity, selecting and cataloguing varieties according to their superiority and above all to construct an interspecific hybridization strategy.

The information generated from this study will be used to identify the germplasms, to provide more evidence on the extent of genetic diversity among the selected cultivars, to determine the phylogenetic relationships among them and most importantly to evolve systematic breeding strategies to improve the yield components of the genotypes.

MATERIALS AND METHODS

Plant Materials

Seeds of five cultivars of mungbean and four cultivars of urdbean (Table 1) were procured from Pulses and Oilseed Research Station, Murshidabad, West Bengal. Seeds of each accession were sown and plants were raised in the field. Young and healthy leaves were randomly harvested and bulked from 25 days old plant, washed to free from dirt and dust, and then quickly mopped, dried on blotting sheets. 1 gm of leaf tissue from each cultivar was subsequently used for each DNA isolation experiment.

Table 1. List of the *Vigna* genotypes used for ISSR analysis and the sources from where these have been obtained are given below.

Sl. No.	Sample Code	Selected Genotypes of *Vigna* Savi	Source
1.	S1	*Vigna mungo* (L.) Hepper - Kalindi	Pulses and Oilseed Research Station, Murshidabad, West Bengal
2.	S2	*Vigna radiata* (L.) Wilczek -Panna	Pulses and Oilseed Research Station, Murshidabad, West Bengal
3.	S3	*Vigna mungo* (L.) Hepper -Sarada	Pulses and Oilseed Research Station, Murshidabad, West Bengal
4.	S4	*Vigna radiata* (L.) Wilczek -Sonali	Pulses and Oilseed Research Station, Murshidabad, West Bengal
5.	S5	*Vigna radiata* (L.) Wilczek -Sukumar	Pulses and Oilseed Research Station, Murshidabad, West Bengal
6.	S6	*Vigna mungo* (L.) Hepper - Gautam	Pulses and Oilseed Research Station, Murshidabad, West Bengal
7.	S7	*Vigna radiata* (L.) Wilczek -Samrat	Pulses and Oilseed Research Station, Murshidabad, West Bengal
8.	S8	*Vigna mungo* (L.) Hepper - Sulata	Pulses and Oilseed Research Station, Murshidabad, West Bengal
9.	S9	*Vigna radiata* (L.) Wilczek -Bireswar	Pulses and Oilseed Research Station, Murshidabad, West Bengal

Genomic DNA Extraction and ISSR-PCR Reaction

Total DNA was extracted from the above mentioned leaf tissue following the CTAB method described by Saghai-Maroof *et al*. (1984) [12] with minor modifications. After isolation, the DNA was quantitified spectroscopically and

visualized under a UV light following electrophoresis on a 0.8% (w/v) agarose gel stained by 0.5 µg/ml ethidium bromide. The resuspended DNA was stored in autoclaved ddH_2O. A total number of 10 ISSR primers (Bangalore Genei Pvt. Ltd., Bangalore, India) that produced a higher number of polymorphic and repeatable fragments were selected to amplify genomic DNA and PCR amplifications were carried out in a thermal cycler (Perkin Elmer, Gene Amp thermal cycler 2400) in a final volume of 25 µl, containing 25 ng template DNA, 200 µM each of the four dNTPs, 10 picomoles of primers, 3 mM MgCl2, 2.5 µl Taq buffer (10 mM Tris HCl pH 9.0, 50 mM KCl) and 0.2 Unit Taq DNA polymerase (Bangalore Genei Pvt., Ltd., Bangalore India). The thermo cycler was programmed for an initial denaturation at 94^{o}C for 4 minutes followed by 40 cycles at 94^{o}C for 1 min, annealing at 43^{0}C to 48^{0}C (for different primers different annealing temperatures were used) for 1 minute and extension at 72^{o}C for 2 minutes, followed by one final extension at 72^{o}C for 6 minutes and at last the hold temperature was of 4^{o}C. 10 µl of amplified PCR amplified product was separated by gel electrophoresis on a 1.8% agarose gel stained by ethidium bromide (0.5 μg/ml of gel solution) and photographed with a gel documentation system (Uvi Tec, UK). DNA fragment sizes on agarose gel were estimated by comparing with low range DNA Ruler (Range from 100bp to 5Kb) markers. To test the reproducibility the reactions were repeated at least twice. For each experiment the reproducibility of the amplification products was tested twice using similar reaction conditions at different times. Only those amplification products that consistently appeared in two replications (consensus products) were scored for further analysis.

ISSR Data Scoring and Analysis

In ISSR analysis, the presence or absence of the bands was taken into consideration and the difference in the intensity of the band was ignored. A particular DNA band (locus) which is generated from the genome of one species, but absent of a second species represents a polymorphism. The banding patterns obtained from ISSR gel were used to assign loci for each primer and scored as present (1) or absent (0). The data obtained from the markers were pooled for different analyses. Jaccard's similarity coefficient values [13] were calculated for each pair wise comparison between genotypes and similarity matrix was constructed. To illustrate the genetic relationships among the species, a dendrogram was constructed based on the similarity matrix using unweighted pair group method with arithmetic average (UPGMA) cluster analysis [14]. All analyses were done using the computer package NTSYS-PC ver. 2.00 [15].

RESULTS AND DISCUSSION

The objective of the present study was to assess the extent of genetic diversity and relationships among the 9 cultivars from two species of *Vigna* based on DNA data. Information on the levels and distribution of genetic diversity of any plant species may contribute to the knowledge of their evolutionary history and potential, and is critical to their conservation management and improvement [16]. Cross breeding between genetically different individuals is recommended, rather than involving individual belonging to related genetic group. The evaluation of genetic diversity and construction of genetic maps has been considered desirable for the efficient use of genetic variations in the breeding programme. ISSR analysis reported in the present work could be useful to select parents to be crossed for generating appropriate populations intended for both genome mapping and breeding purposes [7].

A total number of 10 ISSR primers (Bangalore Genei Pvt. Ltd., Bangalore, India) (Table 2) that produced polymorphic and reproducible fragments were selected to amplify genomic DNA of the genotypes under investigation. Ten primers amplified a total number of 624 bands under 104 loci across nine genotypes with average of 10.4 loci / primer (Figure 1a and Figure 1b). Of the total 104 loci scored in the nine cultivars with different primers, 80 were polymorphic. Therefore, the cultivars exhibited an overall polymorphism of 76.92%. The total number of the amplified loci produced by each primer varied from a minimum number of 10 by primer Oligo- 01, Oligo- 02, Oligo- 03, Oligo- 04, Oligo- 08 and Oligo- 10 to a maximum of 11 by primer Oligo- 05, Oligo- 06, Oligo- 07 and Oligo- 09. The percentage of polymorphism ranged from 50% (primer Oligo- 10) to 100% (primer Oligo- 01, Oligo- 07 and Oligo- 08). The size of amplified bands also varied with different primers. As many as eight primers out of ten showed 60% or more polymorphism. In general, the extent of polymorphism found was high enough. The data obtained was subjected to UPGMA analysis to find out the relationship between the cultivars analyzed. The value of Jacaard's similarity coefficient ranged from 0.566 to 0.793.

The nine cultivars under investigation were clustered into 5 viz., C1, C2, C3, C4 and C5 with 1, 5, 1, 1 and 1 genotypes respectively (Figure 2) at 56.6% similarity. Cluster C1, C3, C4 and C5 consisted of only one genotype each namely Kalindi, Sarada, Gautam and Sulata respectively. Cluster C2 comprised of 5 genotypes namely Panna, Sonali, Sukumar, Samrat and Bireswar, all of which happens to be cultivars of mung bean.

Table 2. List of ISSR primers and their sequences along with some of the characteristics of the PCR-amplified products obtained in *Vigna* genotypes

Primer Code	Primer Sequence (5' to 3')	Total No. of Amplified Loci	Total No. of Polymorphic Loci	% of Polymorphism
Oligo-01	AGAGAGAGAGAGAGAGC	10	10	100
Oligo-02	AGAGAGAGAGAGAGAGT	10	08	80
Oligo-03	CTCTCTCTCTCTCTCTG	10	09	90
Oligo-04	CTCTCTCTCTCTCTCTA	10	07	70
Oligo-05	ACACACACACACACACG	11	07	63.64
Oligo-06	ACACACACACACACACT	11	06	54.55
Oligo-07	TCTCTCTCTCTCTCTCA	11	11	100
Oligo-08	GAGAGAGAGAGAGAGAC	10	10	100
Oligo-09	GAGAGAGAGAGAGAGAT	11	07	63.64
Oligo-10	CACACACACACACACAG	10	05	50

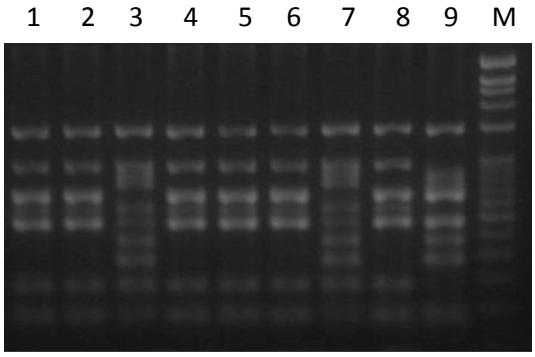

Fig.1a. Ethidium bromide stained 1.8% agarose gel showing PCR-amplified products of 9 Vigna genotypes using the ISSR primer Oligo-04 (5' CTCTCTCTCTCTCTCTA 3'); Lane 1 to 9 correspond to the genotypes listed in Table 1. Lane-M, low range DNA Ruler (Range from 100bp to 5 Kb) marker

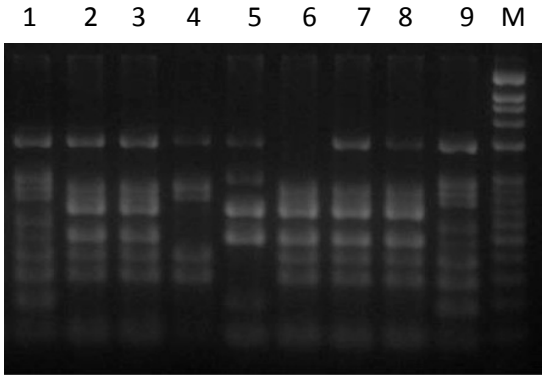

Fig.1b. Ethidium bromide stained 1.8% agarose gel showing PCR-amplified products of 9 Vigna genotypes using the ISSR primer Oligo-08 (5' GAGAGAGAGAGAGAGAC 3'); Lane 1 to 9 correspond to the genotypes listed in Table 1. Lane-M, low range DNA Ruler (range from 100bp to 5 Kb) marker

All the five cultivars of mung bean, clustered under C2, shared a similarity index of 0.724 and exhibited lowest genetic divertsity among the nine cultivars under study. Panna showed the highest genetic diversity among all the five cultivars of mung bean, but was surprisingly and interestingly lower than any of the four cultivars of urd bean. The urd bean variety Kalindi seems to be the genetically most close one to the mung bean varieties studied. Cluster C4 consisted of the single genotype Gautam was significantly different from all other genotypes used in the present investigation. In comparison to other cultivars, Gautam showed the most genetic variability, suggesting utilization of this genotype over others for breeding programme and in transferring important characters into different cultivars. Overall, a high degree of genetic diversity among the 9 cultivars was recorded, which can be used for crop improvement especially keeping in mind the fact that mung bean has been reported to cross with a number of *Vigna* species when it is used as a female parent.

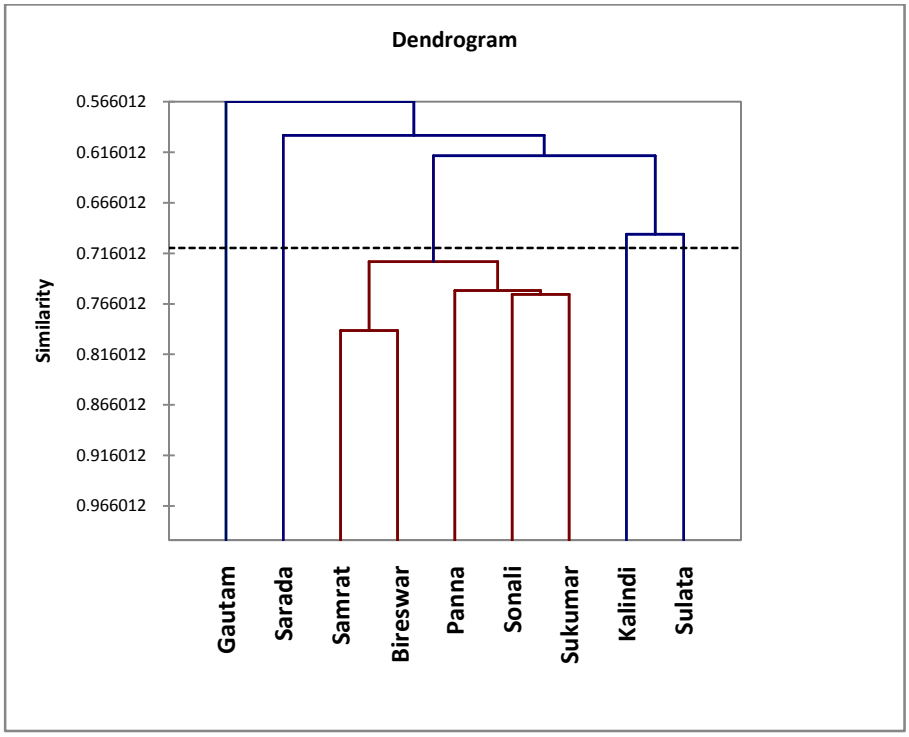

Figure 2. Dendrogram, generated using UPGMA analysis, showing the genetic relationships among the 9 cultivars of two *Vigna* species based on ISSR markers

The assessment of nature and extent of genetic diversity and identification of nine different cultivars were done by ISSR analysis. The obtained results indicated that ISSR marker system can be effectively used in determination of genetic relationship among *Vigna* cultivars. ISSR markers successfully revealed a remarkable molecular discrimination of the cultivars under study. All samples could be identified and distinguished from one another based on the banding patterns generated through PCR. ISSR markers are useful in the assessment of genetic diversity, through detection of duplicate samples in germplasm collection, and the selection of a core collection to enhance the efficacy of germplasm management for use in breeding and conservation programmes. The genetic diversity obtained in this study might be useful in future strategies for evolution of desired genotypes.

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