

Assessment of molecular genetic diversity in some green gram cultivars as revealed by ISSR analysis

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

ABSTRACT

Molecular marker is a useful tool for assessing genetic variation and resolving cultivar identities. The objective of the present study was to assess the extent of genetic diversity and reveal the phylogenetic relationships among the 5 green gram 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 mung bean genotypes under investigation. Ten primers amplified a total number of 353 bands under 93 loci across five genotypes. The cultivars exhibited an overall polymorphism of 52.69%. The value of Jaccard's similarity coefficient ranged from 0.724 to 0.793. The five mung bean cultivars were grouped into 3 clusters at 72.4 per cent similarity. In comparison to other cultivars, Panna showed most genetic variability, suggesting utilization of this species over others for breeding programme and in transferring the characters into different mung bean cultivars. Overall a moderate degree of genetic diversity among the mung bean cultivars was recorded. Nevertheless, the reasonable diversity observed in this study may be exploited for further yield improvement.

Key words: Molecular marker; genetic diversity; green gram; ISSR; polymorphism.

INTRODUCTION

Legumes represent the third largest family of flowering plants with 730 genera and over 19,500 species [1]. *Vigna Savi*, belonging to the family Fabaceae is a large pan tropical genus with 104 species [2]. *Vigna radiata* (L.) Wilczek, commonly known as mung bean or green gram, is an important legume and occupies pivotal position in Indian Agriculture. It is thought to have originated in the Indian subcontinent [3].

Mung bean is an essential crop in developing countries where it is consumed as dry seeds, fresh green pods or leaves because of its high protein, vitamin and mineral content. It is also a very important crop because of its adaptation to short growth duration, low water requirement, and ability to increase soil fertility and usefulness in crop rotation practices also. Other properties like easy digestibility and low proportions of flatulence factors also add to its value among the pulse crops. Pulses are an essential source of dietary protein particularly in the predominantly vegetarian population of Indian subcontinent.

However, the global productivity of pulses in general, and mung bean in particular, is very low as compared to cereals. During the last five decades, annual pulse production in India has witnessed only a marginal increase (from 12.70 million tonnes in 1960–1961 to 14.6 million tonnes in 2008–2009) [4]. 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, the development of sustainable resistance with higher yields has not been achieved due to the narrow genetic diversity in the gene pool of the present cultivars [5]. It is imperative to further explore the genetic diversity available in this crop for better utilization of genetic resources in yield improvement.

Over the years, the methods for detecting and assessing genetic diversity have extended from analysis of discrete morphological traits to molecular traits. Characterization and cataloguing of germplasm have been traditionally carried out by using morpho-agronomic traits. However, these traits may not be significantly distinct and usually require growing plants to maturity prior to identification. Moreover, morphological characters may be unstable due to environmental influences. The morphological markers were not quite enough to expose the genetic diversity and do not reflect real genetic relationships. Molecular markers based on the DNA sequence are more varied and reliable. They are unaffected by environment and detectable in all stages of development. Molecular marker is a powerful tool that can yield significant information and enhances the scope of using a germplasm in the crop improvement programmes. Among the several DNA marker systems that are now being used in diversity studies of plants, inter simple sequence repeat (ISSR) [6] 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 [7]. 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 [7].

The efficiency and effectiveness of conventional breeding can be significantly improved by using molecular markers. Genetic variability and divergence is an important tool for any breeding programme. 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 from West Bengal using inter simple sequence repeat (ISSR) marker. 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 Material

Seeds of five different cultivars of green gram (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 mung bean cultivars used for ISSR analysis and the sources from where these have been obtained are given below

Sample code	Name of the cultivar	Source
MV1	Panna	Pulses and Oilseed Research Station, Murshidabad, West Bengal
MV2	Sonali	Pulses and Oilseed Research Station, Murshidabad, West Bengal
MV3	Sukumar	Pulses and Oilseed Research Station, Murshidabad, West Bengal
MV4	Samrat	Pulses and Oilseed Research Station, Murshidabad, West Bengal
MV5	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-Marooof *et al.* (1984) [8] with minor modifications. After purification, it was quantified spectrophotically and visualized under a UV light after electrophoresis on a 0.8% (w/v) agarose gel stained by 0.5 µg/ml ethidium bromide. The resuspended DNA was stored in autoclaved ddH₂O. A total number of 10 ISSR primers (Bangalore Genei Pvt. Ltd., Bangalore, India) that produced a higher number of polymorphic and reproducible fragments were selected to amplify genomic DNA. 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°C for 4 minutes followed by 40 cycles at 94°C for 1 min, annealing at 43°C to 48°C (for different primers different annealing temperatures were used) for 1 minute and extension at 72°C for 2 minutes, followed by one final extension at 72°C for 6 minutes and at last the hold temperature was of 4°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. 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 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 [9] 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 [10]. All analyses were done using the computer package NTSYS-PC ver. 2.00 [11].

RESULTS AND DISCUSSION

The objective of the present study was to assess the extent of genetic diversity and relationships among the 5 green gram varieties based on DNA data. 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.

A large number of high-yielding cultivars have been released for most of the important crop plants to meet the requirements of diverse agro climatic conditions, agronomic practices, and preferences of the consumers. However, some crops such as mung bean exhibit recalcitrance to such efforts, as it has not been possible to achieve a quantum jump in their productivity. This phenomenon is evident in a vast array of crops wherein a large number of studies have reported a rather narrow genetic base of the improved cultivars. This is due to the fact that different accessions may have common pedigrees, at least for one of the parents and may have been subjected to same selection during their breeding. These observations reveal that in spite of the large collections of diverse germplasms made over the years; few have found their way into the mung bean improvement programmes. Secondly, a tendency to add desirable/improved characters in the already popular and in-use cultivar have been responsible for the repeated use of a few lines as the background material in the breeding programmes, resulting in the subsequent alarmingly narrow genetic base. In addition, a large proportion of alleles of higher productivity have been lost in the present populations of mung bean due to overriding role of natural selection even long after the crop domestication. Further, pulses have been pushed to the marginal conditions in dry lands of low soil fertility because of greater importance of cereals. These factors might be the most likely reasons for the low yields of mung bean cultivars.

A total number of 10 ISSR primers (Bangalore Genei Pvt. Ltd., Bangalore, India) that produced polymorphic and reproducible fragments were selected (Table 2) to amplify genomic DNA of the mung bean genotypes under investigation. Ten primers amplified a total number of 353 bands under 93 loci across five genotypes with average of 9.3 loci / primer (Figure 1a and Figure 1b). Of the total 93 loci scored in the 5 cultivars with different primers, 49 were polymorphic and 15 were unique. Therefore, the cultivars exhibited an overall polymorphism of 52.69%. The total number of the amplified loci produced by each primer varied from a minimum of 6 by primer Oligo-03 to a maximum of 11 by primer Oligo-06. The percentage of polymorphism ranged from 10% (primer Oligo-05 and Oligo-09) to 90% (primer Oligo-08). The size of amplified bands also varied with different primers. Only three out of 10 primers showed 80% or more polymorphism and as many as six primers showed 50% or more polymorphism whereas four primers showed less than 50% polymorphism. In general, the extent of polymorphism found was moderately high.

Table 2. List of ISSR primers and their sequences along with some of the characteristics of the PCR-amplified products obtained in mung bean cultivars

Primer name	Primer Sequence (5' to 3')	Total No. of Amplified Loci	Total No. of Polymorphic Loci	% of Polymorphism
Oligo-01	AGAGAGAGAGAGAGAGC	09	08	88.89
Oligo-02	AGAGAGAGAGAGAGAGT	10	08	80
Oligo-03	CTCTCTCTCTCTCTG	06	01	16.67
Oligo-04	CTCTCTCTCTCTCTA	10	07	70
Oligo-05	ACACACACACACACG	10	01	10
Oligo-06	ACACACACACACACT	11	06	54.54
Oligo-07	TCTCTCTCTCTCTCA	07	03	42.85
Oligo-08	GAGAGAGAGAGAGAGAC	10	09	90
Oligo-09	GAGAGAGAGAGAGAGAT	10	01	10
Oligo-10	CACACACACACACAG	10	05	50

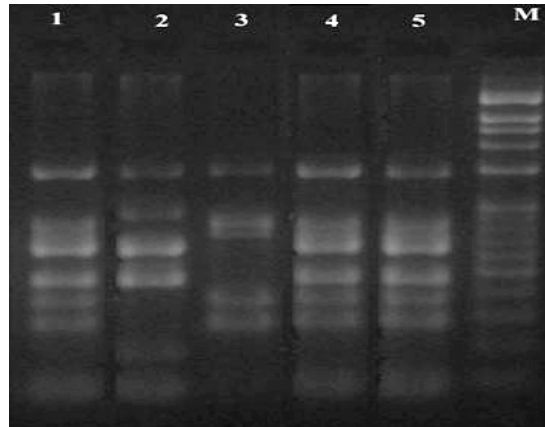


Figure 1a. Ethidium bromide stained 1.8% agarose gel showing PCR-amplified product of 5 green gram cultivars using the ISSR primer Oligo-01 (5'AGAGAGAGAGAGAGC3'); Lane 1 to 5 corresponds to green gram cultivars listed in Table 1. Lane-M, low range DNA Ruler (Range from 100bp to 5Kb) marker

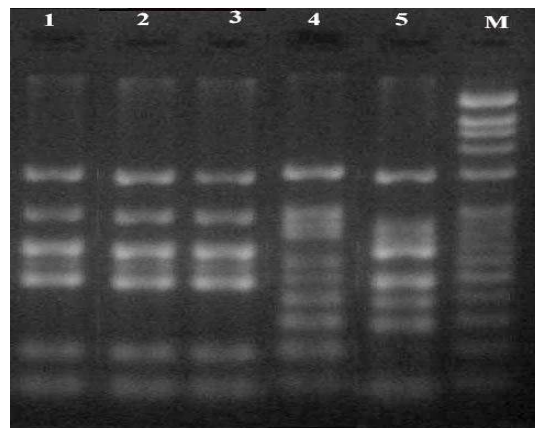


Figure 1b. Ethidium bromide stained 1.8% agarose gel showing PCR-amplified products of 5 green gram cultivars using the ISSR primer Oligo-02 (5'AGAGAGAGAGAGAGT3'); Lane 1 to 5 corresponds to green gram cultivars listed in Table 1. Lane-M, low range DNA Ruler (range from 100bp to 5Kb) marker

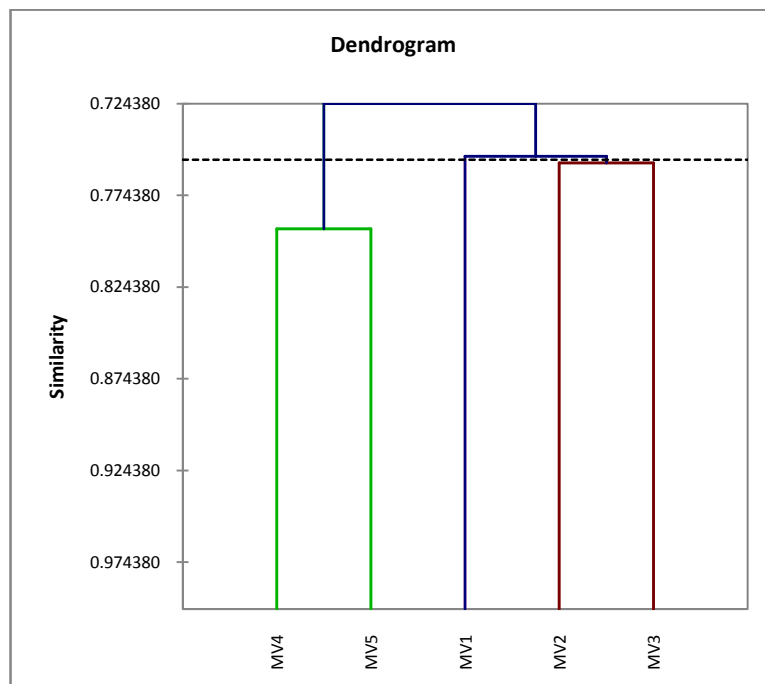


Figure 2. Dendrogram, generated using UPGMA analysis, showing the genetic relationships among the 5 cultivars of green gram based on ISSR markers

The data obtained was subjected to UPGMA analysis to find out the relationships among the cultivars being analyzed. The value of Jaccard's similarity coefficient ranged from 0.724 to 0.793. The five mung bean cultivars were clustered into 3 viz., C1, C2, C3 with 1, 2 and 2 genotypes respectively (Fig.2) at 72.4 per cent similarity. Cluster C1 consisted of only one genotype namely Panna. Cluster C2 comprised of 2 genotypes namely Sonali and Sukumar. Cluster C3 comprised again of 2 genotypes namely Samrat and Bireswar.

In comparison to other cultivars, Panna showed most genetic variability, suggesting utilization of this species over others namely Samrat, Bireswar, Sonali and Sukumar for breeding programme and in transferring the characters into different mung bean cultivars. The cultivar Sonali showed most genetical similarity with Sukumar and formed a cluster. The genotype of Samrat, showed most genetical similarity with Bireswar. The cultivar Panna showed more closeness with Sonali-Sukumar cluster, as shown by high values of similarity index between them. Overall a moderate degree of genetic diversity among the mung bean cultivars was recorded. Nevertheless, the reasonable diversity observed in this study may be exploited for further yield improvement. Collection of diverse germplasm from centres of diversity and from geographically distant sources may broaden the genetic base. The genetic base could also be broadened through the use of radiation, which is a drastic method of mutagenesis resulting in a major reshuffle of the genome [12]. The obtained results indicated that ISSR marker system can be effectively used to identify and differentiate the mung bean cultivars, 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 in order to improve the yield components of the genotypes.

Acknowledgements

Authors are grateful to the Head, Department of Botany, University of Kalyani, West Bengal for providing central equipment facility funded by DST-FIST. and Dr. Amitava Dutta, Scientist, Pulses and Oilseed Research Station, Murshidabad, West Bengal for kindly providing the seeds.

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