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Diversity analysis by RAPD and ISSR markers among the selected mungbean [*Vigna radiata* (L.) Wilczek] genotypes

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Abstract

Narrow genetic base in the working collection of mungbean in India is a bottleneck in the improvement of this crop. Present study gave emphasis for making sizable, but effective working gene pool. Molecular markers, like RAPD and ISSR were employed to assess the genetic diversity among selected germplasm comprising varieties, landraces and wild accessions. Though polymorphism among the varieties was moderate, it was high (83%) when the whole set of germplasm was considered. One mungbean variety, PS-16 with determinate growth habit and a wild accession, Sub-14 (*V. radiata* var. *sublobata*) was found most diverse as revealed from the lowest Jaccard's similarity coefficient value (0.34). One of the landraces from West Bengal, A-5 (*Chinnimung*) was also found distantly located in dendrogram from all varieties and other landraces. The efficiency of ISSR markers over RAPD markers was well visualized from higher frequency polymorphic bands and polymorphic information content values.

Key words: Mungbean, RAPD, ISSR markers, genetic diversity

Introduction

Mungbean [*Vigna radiata* (L.) Wilczek] is a widely cultivated pulse crop in Asian countries. The properties like easy digestibility and production of low flatulence factors make this crop gradually acceptable by the people of Africa, Latin America and Australia. Therefore, the economic importance of this crop is slowly rising. It was established earlier through morphological evaluation that one of the main constraints for lack of breakthrough in mungbean production has been the lack of genetic diversity in the working gene pool [1]. Therefore, utilization of more diverse genotypes is urgently needed for improvement of yield and quality as well as for imparting disease and pest resistance. Information regarding DNA polymorphism is more elaborative than phenotypic level to properly explain the genetic diversity and to find out more diverse genotypes for a crossing programme. DNA fingerprinting in some selected varieties of mungbean in India has been done through RAPD markers by Lakhanpaul *et al.* [2] and AP-PCR markers by Saini *et al.* [3], Recently, Inter-Simple Sequence Repeat (ISSR) marker was found simpler and capable of producing reproducible and greater polymorphism than other marker systems [4]. ISSR is an alternative method of SSR where the repeated sequences are used as primers to produce polymorphism. It was found more effective in closely related crops, like blackgram [5]. In this context, the present investigation was framed to assess the genetic diversity present in a working collection through molecular markers and also to make a comparison between the efficiency of ISSR and RAPD marker system for evaluating mungbean germplasm.

Materials and methods

Twelve genotypes of mungbean (Table 1) comprising of six released high yielding varieties, four aromatic land races and two wild accessions (*Vigna radiata* var. *sublobata*) having pest and disease resistance, were subjected to RAPD and ISSR analysis (Table 1). These could be considered as representative genotypes likely to be used in the future mungbean-breeding programme in Eastern India. Genomic DNA was extracted from 100 mg of tender leaves from 3-5 days old seedlings of each entry according to the procedure described by Bhattacharyya and Mandal [6]. Ten primers each of RAPD and ISSR were synthesized and employed in the study. Five each of RAPD and ISSR primers were selected based on their capability of giving distinct reproducible bands, confirmed by repeating the PCR reaction thrice. RAPD reaction was performed with the selected primers, namely, RA_1 (AGC GCC ATT G), RA_3 (GTC GCC GTC A), RA_{11} (TGG TCG CTG A), RA_{22} (TGG TCA CGG A) and RA_6 (GTG ATC GCA G) following the method of Williams *et al.* [7] with a little modification [6]. For ISSR reaction [4] the selected primers were IS-61:$(GA)_8$ T; IS-62: $(GATA)_4$; IS-63: $(AG)_8$ C; IS-64: T $(TTA)_4$ TT and IS-65: $(AG)_8$ T. The 25 µl PCR reaction mixture contained 4 µl 2.5mM dNTP, 20 ng DNA template, 2.5 emul 10x Taq polymerase assay buffer, 100ng primer and 1.5U Taq polymerase enzyme (purchased all from Genei, Bangalore, India). PCR products were analyzed on a 1.5% agarose gel in Ix TBE buffer (100 mM Tris-HCl,

Table 1. Selection of germplasm of mungbean for this study

No.	Germplasm	Seed colour	Important characters
Released varieties			
1.	NP-28	Green	Early flowering, High protein (Roy, 2003)
2.	B-27	Yellow	High yield, high protein,more sulphur containing amino acid (Roy, 2003)
3.	SML-286	Green	Early maturing, high yield (Roy, 2003)
4.	PS-16	Green	Dwarf, erect, high protein, high yield (Singh, 1991)
5.	Kopergaon	Dull black	High yield (Singh, 1991)
6.	BM-18	Brown	Early maturing (Roy, 2003)
Local germplasm and their selection			
1.	Midnapur local	Dull black	Early maturing, semi dwarf, scented
2.	Malda-95-13	Yellow	Late flowering, yellow seeded, long pod, scented
3.	Dantan sonamung	Green	Late flowering, scented
4.	A-5 (Chinni mung)	Dull black	Sweet and scented
Wild accessions (*Vigna radiata* var *sublobata*)			
1.	Sub-2	Dark brown	Brown seeded, medium early, resistant to yellow mossaic virus and bruchid
2.	Sub-14	Green	Spreading, long flowering period, late maturity, purple leaf colouration, lobbed leaf, resistant to YMV and Bruchid

pH 8.3; 0.8 mM Boric acid; ImM EDTA) at 60V. Bands were visualized by ethidium bromide staining (0.5 µg/ml) and photographed on trans-illuminator.

Bands on RAPD and ISSR gel were scored as present (1) and absent (0) in the data sheet. Data were analyzed and similarity matrix was constructed from binary data with Jaccard's coefficients [8] and dendrograms were generated with unweighted pair-group method arithmetic average (UPGMA) algorithm, using NTSYS-pc Version 2.02 software [9].

Results and discussion

The amplified fragment size of PCR products using ISSR and RAPD primers, varied from 200 bp to 2200 bp. This set of germplasm manifested high level of genetic diversity, realized from higher percentage of polymorphic bands using molecular markers (83%). Among the selected germplasm, varieties also manifested moderate level of polymorphism (65.9%) while comparing with local collection (67.4%) and wild accessions of mungbean (57.14%) employing same set of primers. The level of polymorphism indicated that among the selected varieties the moderate level of diversity was present as they originated from different states of India occasionally utilizing local and exotic gene pool. This was supported by the fact that cv. Kopergaon was a selection from landrace of Maharastra

Table 2. Information regarding polymorphism

Polymorphism parameters	Varieties	Local germ-plasm	Wild access-ions	RAPD	ISSR	Total
Percentage of polymorphic bands	65.9	67.4	57.14	70.0	90.91	83.0
Average bands/primer	5.5	5.38	4.375	5.0	8.25	6.625
Average polymorphic bands/primer	3.63	3.63	2.5	3.5	7.5	5.5
Average PIC	0.181	0.193	0.192	0.177	0.343	0.26

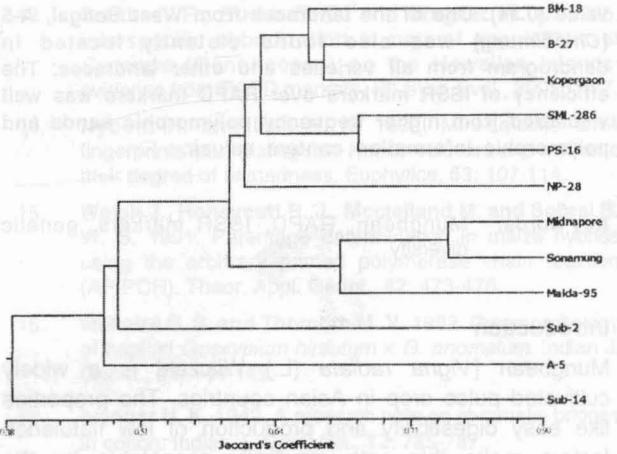

Fig. 1. Dendrogram of genetic distance of 12 germplasm of mungbean constructed from RAPD and ISSR markers

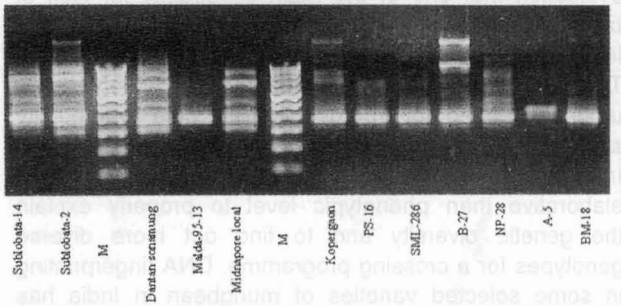

Fig. 2. ISSR profile of 12 mungbean genotypes obtained by IS-61, a GA repeat motif: $(GA)_8$ T

whereas PS-16 was a selection of introduced germplasm from Iran [10]. Average polymorphic bands/primer was same (3.63) for varieties and local collections while it was 2.5 for wild relatives. But from the Polymorphism Information Content (PIC) values, the genetic diversity among the selected germplasm was found to be comparatively higher (0.26) than the varieties (0.18), wild accessions (0.19) and local collections (0.19), separately (Table 2). No definite inferences could be drawn from this observation as the sample size of local collection and wild accessions was small. Lakhanpaul *et al.* [2] found low to moderate polymorphism while analyzing 32 Indian mungbean cultivars using 21 RAPD primers. Broadly, there were four clusters in the dendrogram (Fig. 1). All the varieties and landraces except, A-5 were grouped in one cluster whereas two wild accessions and A-5 (*Chinnimung*) were placed distantly from each other and also from the other germplasm. Jaccard's similarity coefficient was the highest between *Midnapore local* and *Dantan sonamung* (0.81) while it was lowest between *Malda-95-13* and Sublobata-14 (0.32). Sublobata-14 was found to be the most diverse followed by a landrace, A-5 (*Chinimung*) and Sublobata-2. Kaga *et al.* [11] found the largest intra-specific variation in *Vigna radiate* with wild forms (*Vigna radiate* var. *sublobata*) through RAPD analysis. In spite of different seed coat colour, high similarity coefficient (>80%) between two local cultivars, *viz.*, *Midnapore local* and *Dantan sonamung*, pointed towards the fact that they had common ancestry and small differences might be due to minor mutations and selection. As a whole, the genotypes selected for this study on the basis of presence and absence of some desirable traits (data not presented) was found quite diverse through molecular analysis.

ISSR markers gave higher polymorphic bands (90.91%) as compared to RAPD markers (70%). Average polymorphic band/primer was also higher for ISSR markers (7.5) than RAPD markers (3.5). Moreover, mungbean germplasm revealed higher level of genetic diversity employing ISSR markers (PIC-0.34) whereas it was much lower (0.18) when RAPD markers were used. Among the RAPD markers, RA3 was having the highest PIC values (0.37) followed by RA_{22} (0.18). Among the ISSR markers, IS-65 (AG repeat) revealed highest genetic differences (0.37) among the germplasm followed by IS-61 (0.35) (Fig. 2) and IS-62 (0.34). Cluster analysis results from RAPD and ISSR data produced similar but not identical phylogenetic relationship. Differences between dendrogram based on two markers could be due to the fact that probability of scoring of non-homologous RAPD fragments as identical is higher in RAPD than ISSR. The numbers of RAPD and ISSR markers, evaluated in this study were insufficient to conclude concretely about their effectiveness. However, dendrogram based on ISSR data correlates better with the phenotypic information of the germplasm than RAPD data (data not presented). Further work to standardize the molecular marker systems for evaluating more mungbean germplasm of diverse origin is underway.

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