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Short Communication

Diversity among selected blackgram accessions on the basis of RAPD and ISSR markers

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Blackgram [*Vigna mungo* (L.) Hepper] commonly known as Kalai or Urd bean is originated in India and was domesticated from *Vigna mungo* var. *sylvestris*. It is an important grain legume for South and South-East Asia and India is most important producer of all. Besides, being used as food for inexpensive source of dietary protein it is better to use for bean sprouts than mungbean for its longer shelf life. In India it is grown both in winter and summer as monocrop and intercrop, respectively. That's why no single plant type is appropriate for all production systems [1]. So the variability among the existing germplasms or the accessions is the primary need to develop appropriate plant type for specific production system. The lack of knowledge on characterization and classification of the considerable diversity present among Indian collections results in poor exploitation of these plant resources. Genetic diversity analysis is traditionally done based on difference in morphological characteristics, which is mostly influenced by environmental factors. Studies based on seed proteins and isoenzymes are not so efficient owing to lack of polymorphism as well as being influenced by environment, source tissue and plant developmental stages.

Genetic diversity assessment of numerous crops has been conducted with DNA marker alone or in tandem with morphological analysis to cross the limitation of morphological-parameters as a source of estimating variability. RAPD and ISSR marker systems was selected as because among all of the marker systems, the technical simplicity and speed of RAPD is main advantage where as ISSR marker was found simpler and capable of producing reproducible and greater polymorphism than other marker system. Keeping all these in mind, the present experiment was conducted (i) to use RAPD and ISSR analyses to estimate the level of genetic similarity among selected blackgram accession which would help to identify accessions of potential relevance for pulse improvement programme, (ii) to identify suitable primer(s) for both the marker systems to study diversity in blackgram.

Seeds of fifty accessions of blackgram was obtained from NBPGR, New Delhi. Genetic diversity was firstly analyzed based on seven morphological characters (in two seasons) and then cluster analysis (data not given) was done following the Tocher's method in both the year. Finally, twenty accessions (Accession Nos. IC37978, IC45208, IC45373, IC53020, IC56048, IC59702, IC61063, PLU508, IC84206, IC106088, PLU277, IC214844, PLU166, IC214845, PLU171, PLU273, PLU406, PLU570, PLU874, PLU648, T9 as a national check variety) were selected. Genomic DNA was extracted from 100mg of tender leaves of 3-5 days old seedlings following the procedure as described earlier [2]. The RAPD reaction were set up with 20 blackgram accessions along with a released variety (T9) using ten different decamer RAPD primers and five different ISSR primers which were selected based on earlier results of amplifying *Vigna* DNA [3]. Finally, four RAPD primers and three ISSR primers were selected (Table 1) based on capability of giving distinct reproducible bands confirmed by repeating the PCR reaction [3] twice. PCR products were resolved on 1.5% agarose gel in 1X TBE buffer at 60 V. Based on gel, bands were scored as present (1) and absent (0) in data sheet. Similarity matrix was constructed from binary data with Jaccard's coefficient and dendogram were generated with UPGMA algorithm using NTSYSpc-version 2.01 software [4].

Reproducible DNA fragments ranging from 200 bp to 2000 bp were taken into consideration for scoring. A total of 95 bands and 50 bands were amplified with the 10 RAPD primers and 5 ISSR primers, respectively. The individual primer produced band between 8(RA_2) to 12 (RA_1) in RAPD where as it was 7 (IS 65) to 13 (IS 63) in ISSR. Out of 95 bands 83 bands in RAPD and out of 50 bands 40 bands in ISSR were found to be polymorphic for one or more accessesions. Percentage of polymorphic bands was higher in RAPD marker (87.4%) than ISSR marker (80%). Average

polymorphic band/primer was also higher for RAPD marker (8.3) than ISSR marker (8.0). The RAPD primer RA_1 amplified maximum number of polymorphic bands (10) and total bands (12) amplified by this primer was also maximum. Bands amplified by primers RA_2 and RA_3 are all polymorphic in nature although total bands amplified by RA_2 was least in RAPD where as in case of ISSR marker, total number of bands amplified by IS63 was maximum (13) but highest number (10) of polymorphic bands were produced by primers, IS63 and IS61.

Regarding the polymorphism, studied blackgram accessions revealed same level of genetic diversity (Table 1) employing both RAPD marker [Polymorphic information content (PIC-0.294] and ISSR marker (PIC-0.291). Among RAPD primers RA_2 was having the highest PIC value (0.347) followed by RA_3 (0.331). Among the ISSR markers IS-61 (GA_8T) revealed highest genetic difference (0.4) among the accessions followed by IS63 (AG_8C)(0.289) and IS65 (AG_8T) (0.183). Considering the higher PIC value and marker index (MI) value it is concluded that two RAPD primers RA_2 and RA_3, and two ISSR primers IS61 and IS63 can be used to estimate the genetic diversity of blackgram germplasms. But further study with more number of genotypes is to be done for confirmation.

Table 1. Sequences of the RAPD and ISSR primers having high polymorphic information content (PIC) value.

Name of the primer	Sequences	PIC value	MI value
RA_1	TGCGGCTGAG	0.227	0.87
RA_2	GTCGCCGTCA	0.347	2.23
RA_3	GTGTGCCCCA	0.331	1.20
RA_4	TGGTCGCTGA	0.273	0.80
RAPD	Average	0.294	
IS61	$(GA)_8T$	0.400	2.57
IS63	$(AG)_8C$	0.289	1.98
IS65	$(AG)_8T$	0.183	0.40
ISSR	Average	0.291	

It was clear from dendogram (Fig. 1) that the accessions under study were more or less similar in genetic make up because all the studied accessions have 70% similarity.

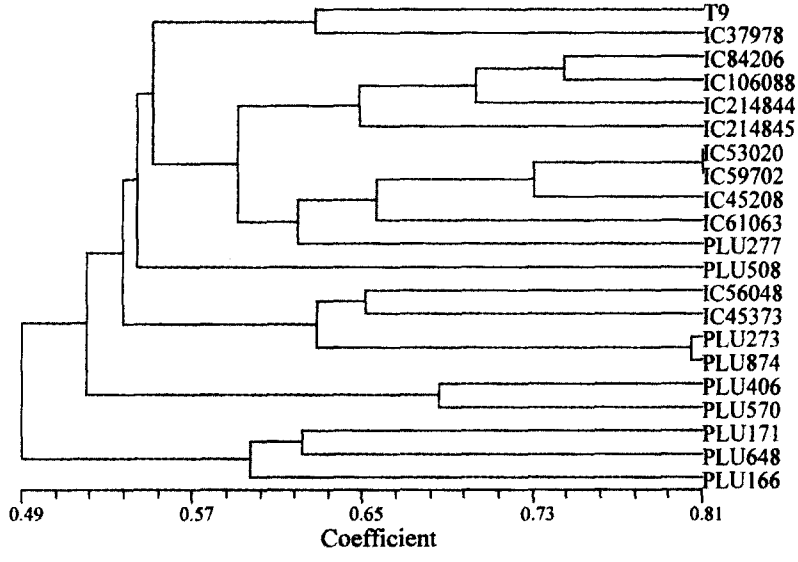

Fig. 1. Dendrogram of selected accessions of blackgram based on RAPD and ISSR data

Besides, dendrogram showed 0.49 as lowest value of Jaccard's coefficient that means two most diverse varieties are also almost 50% similar. Accessions like PLU166, PLU648 and PLU171 could be fruitfully used for the improvement, as they are most diverse in relation to a popular high yielding variety, T9. Identified four primers can be of significant interest in varietal identification by molecular technique especially where cost of amplification is an important factor.

References

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