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European Journal of Experimental Biology, 2014, 4(2):30-34

ISSN: 2248 –9215
CODEN (USA): EJEBAU

Analysis of genetic diversity in some black gram cultivars using ISSR

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ABSTRACT

The objective of the present study was to assess the extent of genetic diversity and the relationships among the 4 black 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 urd bean genotypes under investigation. Ten primers amplified a total number of 269 bands under 103 loci across four genotypes. The cultivars exhibited an overall 65.05% polymorphism. The value of Jacaard's similarity coefficient ranged from 0.557 to 0.697. The four urd bean cultivars were grouped into 3 clusters at 55.7 per cent similarity. In comparison to other cultivars, Gautam showed most genetic variability, suggesting utilization of this species over others namely Sulata, Kalindi and Sarada for breeding programme and in transferring the characters into different urd bean cultivars. Overall, a high degree of genetic diversity among the urd bean cultivars was recorded. It could be concluded that black gram has sufficient amount of genetic diversity and a wide range in genetic base of the studied genotypes which can be used for crop improvement.

Key words: Genetic diversity; black gram; ISSR; polymorphism; crop improvement.

INTRODUCTION

Vigna mungo (L.) Hepper, commonly known as urd bean or black gram or mash, is a grain legume domesticated from *V. mungo* var. *sylvestris* [1]. Urd bean originated in India [2] and is now widely cultivated in the Indian subcontinent as well as to a lesser extent in Thailand, Australia and other Asian and South Pacific countries [1].

Black gram is one of the important pulse crop grown in a wide range of agro-climatic conditions of the country. It is cultivated in all the seasons in India. During *kharif* season, it is grown as a sole crop or mixed with sorghum, pearl millet and pigeon pea. In *rabi* and *zaid* (summer) seasons, it is cultivated as a pure culture [3]. It is cultivated as follow-up crop after rice cultivation [1]. However, its productivity is very low and the major constraints in achieving higher yield of this crop are low yield potential and narrow genetic base of existing cultivars, absence of suitable genotypes for different cropping system, poor harvest index and susceptibility to diseases [4], [1]. Lack of suitable varieties and genotypes with adaptation to local condition is among the factors that also affects the production.

It is an excellent source of easily digestible, good quality protein. It forms one of the important constituents in the dietary practices of the local population and is affordable due to lower price than that of other pulses. However, the global productivity of pulses in general, and urd bean in particular, is very low as compared to cereals. India is the largest producer and consumer of pulses in the world. Slow pulse production growth has substantially reduced the per capita consumption of pulses, especially in predominantly vegetarian countries (from 63.0 g/day in 1961 to 27.3 g in 2010 in India) [5].

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. The molecular markers are a powerful tool that can yield significant information and enhances the scope of using 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, to study genetic diversity, population genetics, taxonomy and phylogeny of many plant species [7]. Identification of different genotypes of crop species is essential when diverse accessions of crop germplasm are to be characterized, newly developed cultivars are to be registered and purity of the variety is to be determined [1]. The evaluation of the genetic diversity would promote the efficient use of genetic variation in the breeding programme. Genetic diversity studies in various cultivars of urd bean using DNA-based marker system are limited to a larger extent.

The objective of the present study was to investigate and compare genetic diversity among four urd bean accessions using inter simple sequence repeat (ISSR). This would help in the identification and differentiation of various cultivars being cultivated, which is especially important for export. The assessment of diversity made would provide us a correct picture of the extent of variation, further helping us to improve the genotypes. The information generated from this study will be used to identify effective strategies for the sustainable management of the genetic resources of black gram.

MATERIALS AND METHODS

Plant Materials

Seeds of four different cultivars of black gram (Table 1) were procured from Pulses and Oilseed Research Station, Murshidabad, West Bengal, India. 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, then quickly mopped and dried on blotting sheets. 1 gm of leaf tissue from each cultivar was subsequently used for each DNA isolation experiment.

Table 1. List of urd bean cultivars used for ISSR analysis and the sources from where these have been obtained are given below

Sample no.	Sample code	Name of the cultivar	Source
1	UV1	kalindi	Pulses and Oilseed Research Station, Murshidabad, West Bengal
2	UV2	sarada	Pulses and Oilseed Research Station, Murshidabad, West Bengal
3	UV3	gautam	Pulses and Oilseed Research Station, Murshidabad, West Bengal
4	UV4	sulata	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*. [8] (1984) with minor modifications. After purification, it was quantified spectroscopically 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 ddH_2O. 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^{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. 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 4 black gram varieties 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 and management [12]. Cross breeding between genetically different individuals is a 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 urd bean genotypes under investigation. Ten primers amplified a total number of 269 bands under 103 loci across four genotypes with average of 10.3 loci / primer (Figure 1a and Figure 1b). Of the total 103 loci scored in the 4 cultivars with different primers, 67 were polymorphic and 34 were unique. Therefore, the cultivars exhibited an overall polymorphism of 65.05%. 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-07, Oligo-08 and Oligo-10 to a maximum of 11 by primer Oligo-05, Oligo-06 and Oligo-09. The percentage of polymorphism ranged from 50% (primer Oligo-04 and Oligo-10) to 100% (primer Oligo-07). The size of amplified bands also varied with different primers. As many as seven primers 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 0.557 to 0.697.

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

Primer name	Primer Sequence (5' to 3')	Total No. of Amplified Loci	Total No. of Polymorphic Loci	% of Polymorphism
Oligo-01	AGAGAGAGAGAGAGAGC	10	06	60
Oligo-02	AGAGAGAGAGAGAGAGT	10	06	60
Oligo-03	CTCTCTCTCTCTCTCTG	10	09	90
Oligo-04	CTCTCTCTCTCTCTCTA	10	05	50
Oligo-05	ACACACACACACACACG	11	07	63.64
Oligo-06	ACACACACACACACACT	11	06	54.54
Oligo-07	TCTCTCTCTCTCTCTCA	10	10	100
Oligo-08	GAGAGAGAGAGAGAGAC	10	06	60
Oligo-09	GAGAGAGAGAGAGAGAT	11	07	63.64
Oligo-10	CACACACACACACACAG	10	05	50

The four urd bean cultivars were clustered into 3 viz., C1, C2, C3 with 1, 1 and 2 genotypes respectively (Figure 2) at 55.7 per cent similarity. Cluster C1 consisted of only one genotype namely Gautam which was significantly different from all other genotypes used in the present investigation. Cluster C2 comprised again of a single genotypes namely Sarada. Cluster C3 comprised of 2 genotypes namely Kalindi and Sulata.

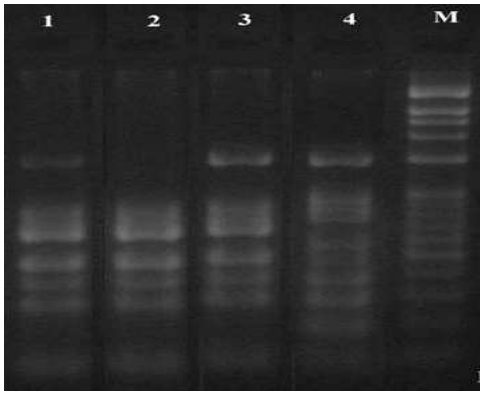

Fig. 1a. Ethidium bromide stained 1.8% agarose gel showing PCR-amplified products of 4 black gram cultivars using the ISSR primer Oligo-01 (5'AGAGAGAGAGAGAGAGC3'); Lane 1 to 4 corresponds to black gram cultivars listed in Table 1. Lane-M, low range DNA Ruler (Range from 100bp to 5Kb) marker

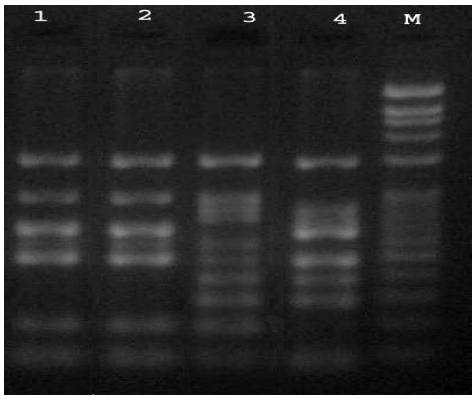

Fig. 1b. Ethidium bromide stained 1.8% agarose gel showing PCR-amplified products of 4 black gram cultivars using the ISSR primer Oligo-02 (5'AGAGAGAGAGAGAGAGT3'); Lane 1 to 4 corresponds to black gram cultivars listed in Table 1. Lane-M, low range DNA Ruler (range from 100bp to 5Kb) marker

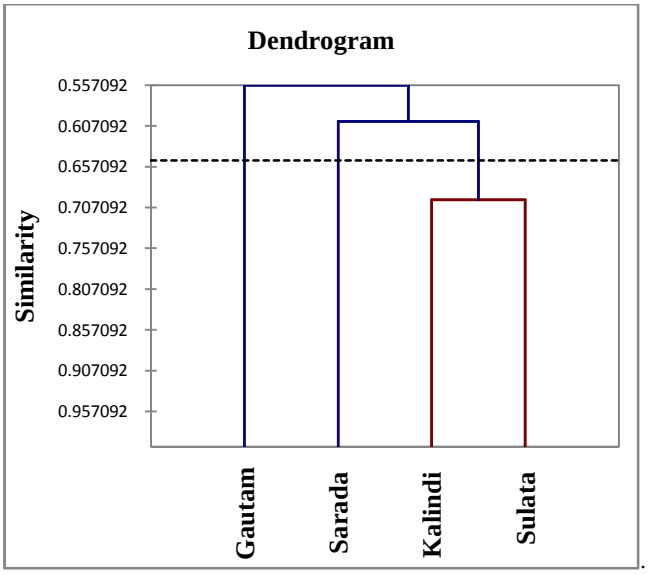

Figure 2. Dendrogram, generated using UPGMA analysis, showing the genetic relationships among the 4 cultivars of black gram based on ISSR markers

In comparison to other cultivars, Gautam showed most genetic variability, suggesting utilization of this species over others namely Sulata, Kalindi and Sarada for breeding programme and in transferring the characters into different urd bean cultivars. The cultivar Kalindi showed most genetical similarity with Sulata and formed a cluster. The cultivar Sarada showed more closeness with Sulata-Kalindi cluster, as shown by high values of similarity index between them. The genotype of Gautam is distantly related with rest of the genotypes of urd bean, as recorded by

low values of the genetic similarity. Overall, a high degree of genetic diversity among the urd bean cultivars was recorded. It could be concluded that black gram has sufficient amount of genetic diversity and a wide range in genetic base of the studied genotypes which can be used for crop improvement.

The assessment of nature and extent of genetic diversity and identification of four different urd bean cultivars were done by ISSR analysis. The obtained results indicated that ISSR marker system can be effectively used in determination of genetic relationship among black gram cultivars. ISSR markers successfully revealed a remarkable molecular discrimination of the four urd bean cultivars under study. All black gram samples could be distinguished from one another based on these polymorphic bands. ISSR markers are useful in the assessment of black gram 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 black gram breeding and conservation programmes. The genetic diversity obtained in this study might be useful in future strategies for evolution of desired 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.

REFERENCES

[1] Srivastava Priya, Anjana Pandey and Diamond Prakash Sinha, *Journal of Plant Breeding and Crop Science*, **2011**, Vol. 3(3), pp. 53-59.
[2] Bhosale U P, B. V. Hallale and S. V. Dubhashil, *Advances in Applied Science Research*, **2013**, 4(3):95-97.
[3] Rama Kant and R.K. Srivastava, *Journal of Food Legumes*, **2012**, 25(1): 1-8.
[4] Chakraborty S, Borah HK, Borah BK, Pathak D, Baruah BK, Kalita H and Barman B, *Notulae Scientia Biologicae*, **2010**, 2: 121-126.
[5] Jitendra Kumar, Arbind K. Choudhary, Ramesh K. Solanki and Aditya Pratap, *Plant Breeding*, **2011**, 130, 297—313.
[6] Zietkiewicz E, A. Rafalshi & D. Labuda, *J. Genomics*, **1994**, 20: 176-183.
[7] Pardhe Deepak D , Satpute Rajendra A, *International Journal of PharmTech Research*, **2011**,Vol.3, No.1, pp 464-470.
[8] Saghai-Maroof M A, Soliman K M, Jorenson R A, and Allard R W, *Proc. Natl. Acad. Sci. USA*, **1984**, 81, 8014-8018.
[9] Jaccard P, *Bull.Soc. Sci. Nat*, **1908**, 44: 223-270.
[10] Sneath P. H. A. and Sokal R, Numerical Taxonomy, Freeman, San Francisco, California, **1973**.
[11] Rohlf FJ, Ntsys-PC, Numerical taxonomy and multivariate analysis system Version II, 80-Setauket, NY, Exeter Software, **1993**.
[12] Karuppanapandian T, Karuppudurai T, Pritam Bala Sinha, Kamarul Haniya, A Manoharan, *African Journal of Biotechnology*, **2006**, Vol. 5 (13), pp. 1214-1219.