P: ISSN NO.: 2394-0344 RNI No.UPBIL/2016/67980 VOL-4* ISSUE-1* (Part-1) April- 2019
E: ISSN NO.: 2455-0817 *Remarking An Analisation*

Assessment of Genetic Diversity in *Canavalia gladiata* using RAPD Markers

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Abstract

RAPD techniques have been found to be highly useful and accurate for the determination of both inter-specific and intraspecific genetic variations in plants. The samples of *Canavalia gladiata* were analyzed for genetic variation using three random primers for each seed samples.The RAPD profile of different accessions belongs to *Canavalia*g enotypes using random decamer primer (OPB-C1, OPB-C2 and OPB-C3**).***Canavalia gladiata* accessions were also grouped into two clusters. Major cluster I consisted of two accessions (IC-44599 and IC-44618B), sharing 82.0% similarity. While IC-44592 was separated as single cluster showing 77.0% similarity with the cluster I.

Keywords: SDS-PAGE, Genetic Diversity, Protein Profiling and UPGMA Cluster Analysis.

Introduction

Seeds from selected accessions of *Dolichos lablab,* were taken, soaked in sterile water and removed seed coats of soaked seeds, and genomic DNA was extracted following the standard method of Krishna and Jowali (1997), with required modification. For genomic DNA isolation, 1.0g of seeds were ground in 10.0ml extraction buffer (100 mMTris-HCl, 50 mM EDTA, 500 mMNaCl, pH 8.0 and 1.0ml of 10% SDS. After thorough mixing, then this mixture was incubated at 60°C for 15 minutes and cooled. Subsequently 5.0ml of Potassium acetate was added and votexed and incubated at 4ºC for 20 minutes. It was centrifuged at 14,000xg for 20 minutes at 4°C. Supernatant was taken out and equal volume of isopropanol and incubated at 4°C for 10 minutes. 700µl of TE (10mM TrisCl, 50mM EDTA, pH-8.0) and 500µl of chloroform: Isoamyl Alcohol (24:1) was added and after mixing thoroughly by vortexing, it was centrifuged at 5,000 rpm for 2 minutes at 4°C. The DNA present in upper aqueous phase was precipitated in one vol. of isopropanol and incubated for 10 minutes at 4°C and it was centrifuged at 10,000 rpm for 5 minutes at 4°C. The DNA pellet was washed twice with 70% alcohol, dried in incubator at 37°C for 30 minutes, and finally, pellet was dissolved in 50µl of TE buffer.

Genomic DNA samples were checked for purity by running it in an agarose gel (0.8%). The quantities of DNA were determined by measuring ratio of absorbance (A) at 260 & 280 nm (A260/A280). 1 µl of a λ DNA marker (10 µg μl^{-1}) was also loaded for visual quantification.Genetic diversity among selected isolates was also assessed by RAPD primer using the 11 RAPD-Primer set purchased from Bangalore Genei, India(Table 1).The presence or absence of individual, distinct and reproducible bands was scored as “1” for presence and “0” for absence. Binary data was pooled and used to construct a composite dendrogram. The software NTSYSpc version 2.02i (Rohlf, 1995) was used to calculate the Jaccard distance coefficient (Jaccard, 1908) and construct the dendrogram using the Unweighted Pair-Group Method with Arithmetic Average (UPGMA).Zymogram is the diagrammatic representation of the enzyme or protein band location in the strip of a gel. It is extrapolated on the basis of relative mobility of each band in the gel. Scoring of bands was done by Rm value and + sign was given for presence of band. A schematic diagram was made for analysis of banding pattern**.**

Aim of Study

In the present investigation, RAPD method of DNA fingerprinting was employed which is widely used in conservation biology because of quick results, cost-effectiveness and reproducibility. DNA profiling (RAPD analysis) of three accessions *Canavalia gladiata*) was carried out using 3 random primers and UPGMA cluster analysis carried to assess the genetic diversity.

Table 1.RAPD primers used for diversity analysis

RAPD Primers	Primer sequence (5'-3')	Total number of bands	No. of Polymorphic Bands	Percent of Polymorphic Bands
OPB-C1	GTTTCGCTCC	7	1	14.2
OPB-C3	CATCCCCCTG	6	2	33.3
OPB-C5	CTCTGGAGAC	4	1	25.0

Review of litrature

Literature review has revealed that a lot of work had been done in other legume crops but three bean genera *Canavalia gladiata*) under present investigation are lagging behind in the race and no similar work keeping all the three genera together been reported any one else in the literature cited. Nagendraraie*t al*. (2011) carried out randomly amplified polymorphic DNA (RAPD) analysis to study genetic diversity among 48 Indian bean (*Lablab purpureus*L. Sweet) genotypes, collected from various parts of India, was analyzed based on morphological traits and randomly amplified polymorphic DNA (RAPD) markers.Kimanie*t al.* (2012) carried out molecular diversity in *Dolichospurpureus* using amplified fragment length polymorphism markers on fifty Kenyan *Dolichos lablab* accessions. Kalidass and Mohan (2010) carried out genetic diversity assessment of wild population of *Mucuna pruriens* (L.) DC. var. utilis using DNA (RAPD) primers genetic diversity assessment of wild population of *Mucuna pruriens* (L.) DC. var. utilis employing five random amplified polymorphic DNA (RAPD) primers. Out of 43 amplified products, 28 showed polymorphism.

Results and Discussion

RAPD techniques have been found to be more useful and accurate for the determination of both inter-specific and intraspecific genetic variations in plants. Similar results have been found by Dikshite*t al*. (2007) for *Vigna umbellata* accessions and estimated 53 per cent of genetic similarity based on RAPD and SSR markers data. In particular, RAPD markers have been successfully employed for the determination of intra-specific genetic diversity in several species (Wolfe and Liston, 1998).

In case of *Canavalia gladiata* accessions IC44599 and IC44618B showed high genetic similarity (similarity coefficient 82%) and IC44592 was in separate cluster and had high similarity with cluster I (similarity coefficient 77%). These results are in conformity of protein profiling results in these genotypes. Ratnaparkhe*et al*.(1995) in *Cajanuscajan* and Maciel*et al*. (2001) in common beans carried out RAPD analysis to reveal high variation in the number of fragments using arbitrary primers and showed that variation may be attributed to the differences in the binding sites throughout genome of the accessions included.Accessions IC44618B, IC44592 and IC44599 in *Canavalia gladiata*were found to be quite distinct and they could be used for their desirable characteristics in breeding programmes for germplasm improvement. The genetic similarities obtained from the analysis can also be used for the selection of the parents to generate mapping populations and for selecting parents for breeding purposes. Similar kind of findings were reported by Weedene*t al*. (1992) in horse gram and Lavanya*et al*. (2008) using RAPD profiles to identify the extent of diversity among 54 accessions of mung bean. Findings in present investigations revealed that significant intra-specific variation was available in the analyzed accessions. The variation in major bands was available in accessions *Dolichos lablab* less in case of *Mucuna* and *Canavalia* species showing maximum similar banding patterns. Conclusions are based upon the analyses of results.

The present study has also shown the importance of the method not only to assess the range of diversity in the germplasm but also to enable potential clustering of accessions on basis of their affinities to each other in agronomic performance of traits and an important identification tool for *Canavalia genotypes*.

Fig. 1(a) RAPD profile of *Canavalia gladiata* accessions using three different primers. Lane 1, 4, 7- IC-44599, Lane2, 5, 8- IC-44618B and Lane3, 6, 9-IC-44592. M- Molecular Marker (range: 250-2000bp)

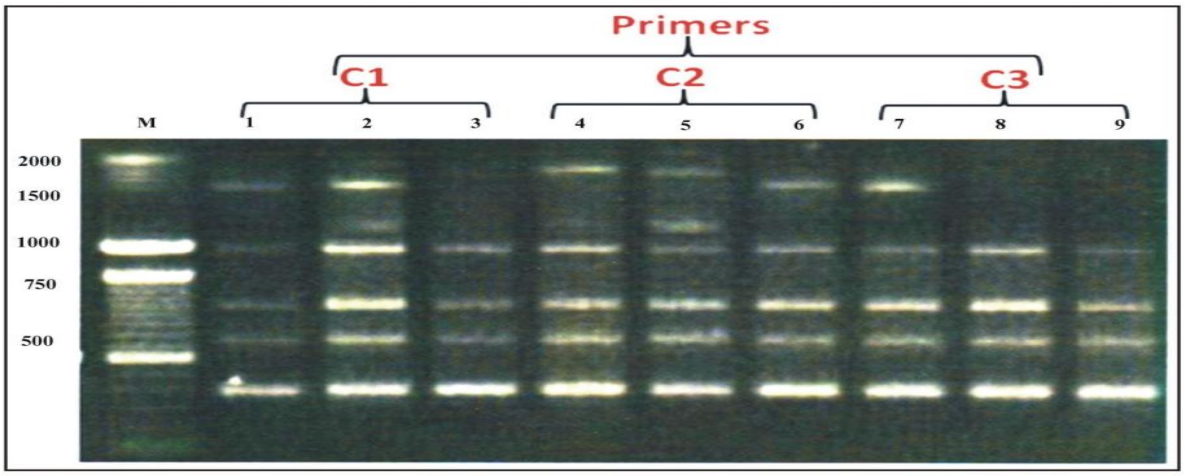
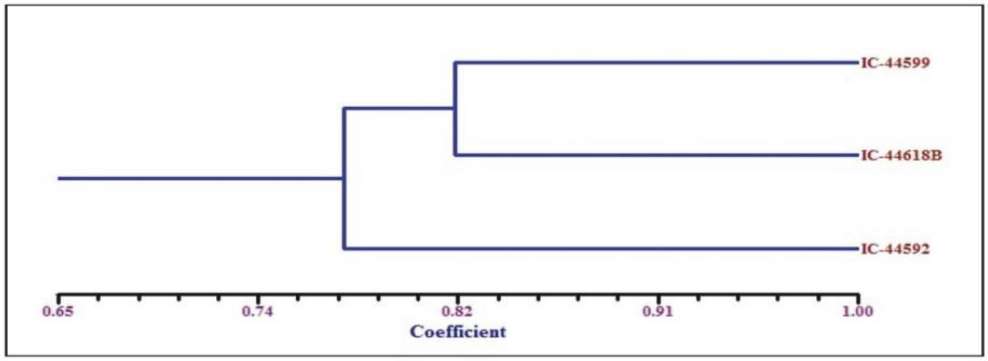


Fig. 1(b)Composite UPGMA dendrogram of *Canavalia gladiata* on the basis of RAPD analysis based on Nei and Li (1979) genetic distances



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