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# Genetic diversity assessment in clusterbean (*Cyamopsis tetragonoloba* (L.) Taub.) by RAPD markers

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## ABSTRACT

**Genetic diversity analysis of 12 clusterbean (*Cyamopsis tetragonoloba* (L.) Taub.) genotypes were carried out using Random Amplified Polymorphic DNA (RAPD) markers. The 19 RAPD primers amplified a total of 212 bands, out of which 151 were polymorphic. The size of amplified DNA fragment varied from 146 to 2995 bp. The polymorphic bands varied from 22.22 percent in OPA-11 to 88.88 percent in OPA-12. Dendrogram based Jaccard's similarity coefficient grouped the 12 genotypes into four major clusters encompassing five subclusters. The first cluster comprised three subclusters with subcluster A1 contained three genotypes viz; GG-2, HG-75 and HG-365. Subcluster B1 had only one genotype GG-1 while, subcluster C1 contained two genotypes *viz*; RGC-471 and HVG-2-30. Cluster 2 entailed two subclusters *viz*; B1and B2 having two genotypes each namely PRT-15 and GAUG-0013 grouped in subcluster B1 and FS-277 and PNB in subcluster B2. The third and fourth cluster contained single genotype GAUG-0522 and GAUG-9404, respectively. The similarity index values ranged from 0.52 to 0.87 indicating the presence of enormous genetic diversity at molecular level. Therefore, RAPD analysis could be used as tool for detecting genetic diversity and can be precisely used for grouping and selection of diverse parents. GG-2, HG-75 and HG-365 (Sub group A1) and GAUG-0522 (Group C) may be utilized for breeding good genotypes with high yield and resistance to bacterial blight in clusterbean.**

***Key words***: *C. tetragonoloba*, Clusterbean, Genetic diversity, RAPD markers.

Clusterbean is an important arid legume known for its adaptation to rugged environments. It has multi facet uses as vegetable, food, fodder and feed. However, its economic importance reflects in having a rubber-like substance called galactomannan in its endosperm that has conspicuous wide arrays of industrial utilities. Lately, the crop assumed enhanced importance due to uses of galactomannan in fracking process of oil exploration (Kyaw *et al.,* 2012; Narayan, 2012).

The genetic variability is the backbone of any breeding programme. More genetically wider is the involvement of parents, better is the chance of recovering high yielding genotypes with appropriate quality and resistance to biotic stresses. The overall expression of different characters is the function of juxtaposition of environment with genotype and this interaction is never consistent making it difficult to have precise selection of the parents. Therefore, it would be advantageous to study polymorphism through molecular markers. Among the various molecular markers, RAPD (Williams *et al*., 1990), are not sequence based and can detect genome wide variation in both coding as well as non-coding region besides being dominant, cheaper and allows a large number of marker to be assayed in short time.

## MATERIALS AND METHODS

### *Plant material and DNA extraction*

Experimental material comprised of twelve genotypes of clusterbean obtained from Centre of Excellence for Research on Pulses, Sardarkrushinagar Dantiwada Agricultural University, Sardarkrushinagar. The genotypes were selected based on different morphological characters and reaction to bacterial leaf blight in particular. The salient features of the selected genotypes included in present study are given in Table 1.

The genotypes were grown in pots. Genomic DNA was isolated from the young leaves as per modified Cetyl Trimethyl Ammonium Bromide (CTAB) method of Murray and Thompson (1980). The quality and quantity of DNA was determined by nano spectrophotometer.

### *PCR and RAPD analysis*

PCR amplification was performed with random decamer primers obtained from Operon Technologies (Almeda, Calif., USA). Amplification was performed in a 25-µl reaction volume containing Taq Buffer B (10X Tris without $MgCl_2$), 25 mM $MgCl_2$, 20 pmol RAPD primer, 50 ng genomic DNA, and 1 U Taq DNA polymerase (Bangalore Genei, Bangalore, India). Amplification was performed in an Eppendorf Master Cycler Gradient (Eppendorf Netheler-Hinz, Hamburg, Germany). Amplification conditions comprised initial denaturation at 94°C for 4 min, 41 cycles at 94°C for 1 min (denaturation), 1.5 min annealing (depending on Tm), 72°C for 2 min (extension) and followed by final extension of 4 min at 72°C. Amplified products were separated on 1.5% agarose gel in 1× TBE buffer (100 mM Tris-HCl, pH 8.3, 83 mM boric acid, 1 mM EDTA) at 50 V. The

gels were stained with 0.5 µg/ ml ethidium bromide solution and visualized by illumination under UV light.

### *Statistical analyses*

Amplified products obtained from random primers were used to estimate genetic distances among the accessions. The entire fingerprint data was converted into a binary matrix based on the presence (1) or absence (0) of individual bands for each genotype. The cluster analysis was performed by using Unweighted Pair Group Methods of Arithmetic Averages (UPGMA) using NTSYS-pc version 2.1 (Numerical Taxonomy and Multivariate Analysis System for Personal Computers, Exeter Software) developed by Rohlf (2000) and analyzed by the SIMQUAL (similarity for qualitative data) program with Jaccard's Similarity Coefficient.

## RESULTS AND DISCUSSION

The role of specific primers as a valuable resource can not be over emphasised in precise assessment of genetic diversity bereft of vague impact of environmental factors. This can be used for efficient selection of diverse parents for efficient crop improvement programme (Virk *et al*., 1995).

The extracted DNA of each genotype was amplified with 19 random decamer primers. A total of 212 bands were obtained with an average of 11.15 bands per primer. Out of these, 151 fragments were found polymorphic. The mean number of polymorphic bands per primer among 12 clusterbean genotypes was 7.94. The size of PCR amplified DNA fragment varied from 146 to 2995 bp. Among the primers, OPA-12 evinced the maximum polymorphism (88.88%), while the lowest polymorphism (22.22 %) was exhibited with OPA-11. The average polymorphism detected was 71.22 % (Table 2) that was good enough for efficient genetic analysis. In consonance to the present findings, Punia et al. (2009) have also reported amplification of maximum number of 20 bands by primer OPB-15 and minimum of 4 bands by primer OPB-1. The average fragments amplified per primer were 11.15 that were also in consonance to the findings of Punia et al. (2009), who had also reported average 10.29 fragments per primer in their study on 18 genotypes of clusterbean.

UPGMA cluster analysis based on Jaccard's Similarity Coefficient grouped the 12 genotypes into four major clusters and five subclusters. The first Cluster A comprised three subclusters with subcluster A1 containing three genotypes *viz.,* GG-2, HG-75 and HG-365. Subcluster A2 had only one genotype GG-1, while subcluster A3 contained two genotypes *viz.,* RGC-471 and HVG-2-30. Second cluster B comprised two subclusters *viz.,* B1 and B2 encompassing two genotypes each i.e. PRT-15 and GAUG-0013 grouped in subcluster B1 and FS-277 and PNB in subcluster B2. The third cluster C and fourth cluster D contained single genotype GAUG-0522 and GAUG-9404, respectively (Fig. 1).

Based on the simple matching coefficient, a genetic similarity matrix was constructed using the RAPD data to assess the genetic relatedness among the 12 accessions. The similarity coefficients ranged from 0.52 to 0.87 for all accessions with the minimum genetic similarity between GAUG-0522 and GAUG-9404 and the maximum similarity between GG-2 and HG-75 (Figure 4). Higher the dissimilarity or diversity between the genotypes, better is the scope to include them in hybridization. Bacterial leaf blight is the major yield limiting factor in clusterbean. Out of the different genotypes studied, GAUG-0522 was resistant to bacterial leaf blight while, GAUG-9404 was susceptible and thereby expected to throw better segregants for resistance to bacterial leaf blight. Sub group A1 contained all the three genotypes *viz.,* GG-2, HG-75 and HG-365 as resistant to bacterial leaf blight. The genetically distant genotype along with resistance to bacterial leaf blight was GAUG-0522 (Group C). Therefore, crosses between three genotypes in Sub group 1A (GG-2, HG-75 and HG-365) and Group C (GAUG-522) could be exploited for enhancing productivity along with resistance to bacterial leaf blight.

The suitability of individual primers for genetic diversity study was determined from the number of polymorphic fragments produced by the different genotypes and the number of them that can be utilized for fingerprinting of individual genotype. This is similar to the method used by and Russell *et al*. (1997) and Rajora and Rahman (2003). Out of the 19 primers used, all but OPA-11 were consistently repeatable and were useful in detecting polymorphism among

**Table 1. List of clusterbean genotypes used for RAPD analysis**

| Genotype | Salient feature | Pedigree |
|---|---|---|
| GG-2 | Resistant to bacterial leaf blight disease | HG 7-4/P2-1 × RGC 137 |
| GAUG-0522 | Resistant to bacterial leaf blight disease | GAUG 90005× HGS 844 |
| HG-75 | Resistant to bacterial leaf blight disease | Selection from germplasm |
| HG-365 | Resistant to bacterial leaf blight disease | Durgajay x Hisar Local |
| RGC-471 | Resistant to bacterial leaf blight disease | Selection from Nagaur district of Rajasthan |
| PRT-15 | Resistant to bacterial leaf blight disease | Not available |
| GG-1 | Susceptible to bacterial leaf blight disease | Mutant of Kutch-8 (10 K r alpha ray's) |
| GAUG- 0013 | Susceptible to bacterial leaf blight disease | HGS 844 × GAUG 9003 |
| GAUG-9404 | Susceptible to bacterial leaf blight disease | Selection from germplasm |
| HVG-2-30 | Susceptible to bacterial leaf blight disease | Pusa Sadabahar x HGS 296 |
| FS-277 | Susceptible to bacterial leaf blight disease | Selection from germplasm |
| PNB | Susceptible to bacterial leaf blight disease | Pusa Mausami × Pusa Sadabahar |

**Table 2. List of primers along with sequences and amplification details**

| Primer | Primer sequence 5' ? 3' | Total number of bands | Number of Polymorphic bands | Per cent Polymorphism |
|---|---|---|---|---|
| OPA-11 | CAATCGCCGT | 9 | 2 | 22.22 |
| OPA-12 | TCGGCGATAG | 9 | 8 | 88.88 |
| OPB-1 | GTTTCGCTCC | 4 | 3 | 75.00 |
| OPB-3 | CATCCCCCTG | 15 | 12 | 80.00 |
| OPB-4 | GGACTGGAGT | 6 | 4 | 66.66 |
| OPB-5 | TGCGCCCTTC | 6 | 3 | 50.00 |
| OPB-7 | GGTGACGCAG | 11 | 8 | 72.72 |
| OPB-13 | TTCCCCCGCT | 13 | 10 | 76.92 |
| OPB-14 | TCCGCTCTGG | 12 | 10 | 83.33 |
| OPB-15 | GGAGGGTGTT | 20 | 17 | 85.00 |
| OPB-16 | TTTGCCCGGA | 14 | 9 | 64.28 |
| OPB-19 | ACCCCCGAAG | 9 | 7 | 77.77 |
| OPH-1 | GGTCGGAGAA | 7 | 5 | 71.42 |
| OPH-8 | GAAACACCCC | 13 | 10 | 76.92 |
| OPH-13 | GACGCCACAC | 15 | 9 | 60.00 |
| OPH-14 | ACCAGGTTGG | 15 | 9 | 60.00 |
| OPH-17 | CACTCTCCTC | 7 | 5 | 71.42 |
| OPH-18 | GAATCGGCCA | 14 | 12 | 85.71 |
| OPH-20 | GGGAGACATC | 13 | 8 | 61.53 |
| **TOTAL** | | **212** | **151** | **71.22** |

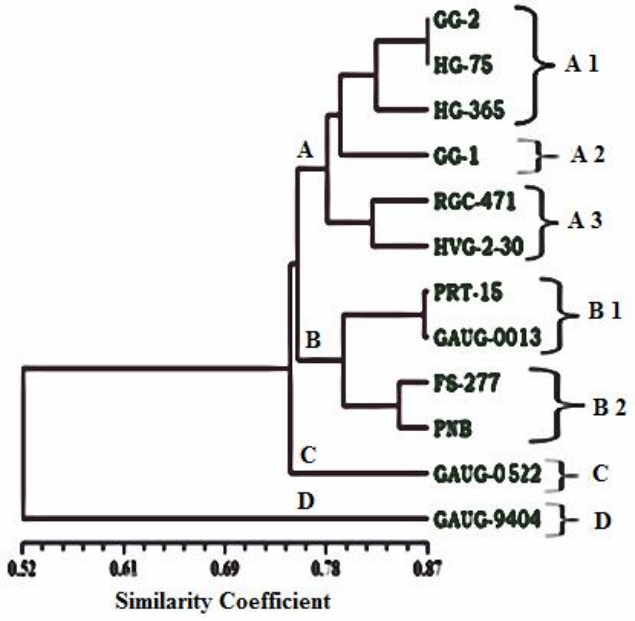


Figure 1. Dendrogram showing clustering pattern of clusterbean genotypes based on genetic similarity values obtained from the RAPD data

the genotypes studied. Further, OPA-12, OPH-8, OPB-15, OPB-14 and OPB-3 were the best primers evincing more than 80 percent polymorphism and encompassed 59 of the 151 polymorphic bands

RAPDs are among the most-widely used markers for economically important traits in cultivated plants. Earlier studies also reported that RAPD technique generates large number of polymorphisms in clusterbean (Pathak et al. 2011). The phylogenetic relationship exhibited among different genotypes of clusterbean in the study was congruent with earlier studies conducted by Punia et al. (2009) and Pathak et al. (2010) Therefore, RAPD analysis could be used as a good tool for detecting genetic diversity and can be precisely used for grouping and selection of diverse parents. From the present study genotypes like GG-2, HG-75 and HG-365 (Sub group 1A) and GAUG-0522 (Group C) may be utilized for breeding good genotypes with high yield and resistance to bacterial blight in clusterbean.

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