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Molecular Characterization of Cluster Bean (*Cyamopsis Tetragonaloba* L Taub.) Using RAPD Markers

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ABSTRACT :Cluster bean commonly known as Guar is an annual legume plant which has a broad spectrum of an important hydrocolloid percent in its endosperms. To determine the level of genetic diversity in relation to geographical origins and morphological characteristics, a total no. of 5 varieties were collected from MPKV, Rahuri and were analyzed using RAPD. Total of 4 operon primers were used which showed amplified 16 amplicons. Out of these 16 amplicons were polymorphic with 33.33 % polymorphism. RAPD data were used to generate the similarity coefficients using Jaccards similarity coefficient software NTSYS-PC. The similarity coefficient values ranged from 0.29 to 0.85, indicating high genetic variability among the selected guar varieties. This information can be used for identification of varieties and further crop improvement programme.

KEYWORDS: Cluster bean, Polymorphism, RAPD, *Cyamopsis tetragonloba*, NTSYS-PC.

I.INTRODUCTION

Cluster bean, (*Cyamopsis tetragomoloba* (L.) Taub) commonly known as Guar, is drought and high temperature tolerant deep rooted summer annual legume of high social and economic significance. It is an important self pollinated, multipurpose and restorative leguminous vegetable crop. It belongs to the tribe Galagae (Indigoferae) of the Leguminosae family. The haploid and diploid chromosome number of all the three genus of species cyamopsis reported to be $n=7$ and $n=14$. Its centre of origin is in the Tropical Africa. The crop is mainly grown in the dry habitats of Rajasthan, Haryana, Gujarat and Punjab and to a limited extent in Uttar Pradesh, Madhya Pradesh, Andhra Pradesh, Tamil Nadu, Karnataka and Kerala. The crop is known for exceptionally high adaptation towards poor and erratic rains, low inputs and less care, soil enrichment properties. The qualities of the crop like high adaptation towards erratic rainfall, multiple industrial uses and its importance in cropping system for factor such as soil enrichment properties, low input requirement. etc have made the guar one of the most significant crops for farmers in arid areas India. Guar is a photosensitive crop and requires specific climatic condition to grow. For proper germination soil temperature ideally should be around 20°C to 25°C, long day period for vegetative growth and short day period for flowering and pod formation. Being a drought tolerant crop it performs well under arid and semi-arid condition. In arid condition Guar grows as rain fed crop which requires 300 to 400 mm rainfall in 3 to 4 spells. It grows under wide range of rainfall from 300 to 550 mm, but heavy rainfall invites many diseases. Guar crop performs better in warm climate and grows well in sub-tropics during summer. It can tolerate high temperature even upto 40°C. The seed is normally Sown in second week of July after rainfall starts. Sowing may get extended upto to August depending on rainfall and is harvested during October and November. It is usually a 90 to 110 days crop. For getting better yield, germination is very important that makes the basic phase of Guar life cycle. Germination is dependent on different factors like seed

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viability, seed size, seed vigour, soil type, soil moisture, soil temperature and relative humidity. Bolder seeds give higher germination compared to small or medium

In India, green and tender pods of cluster bean are used as a favorite vegetable in many parts of the country. It is also grown as a forage crop. Sometimes it is used in reclamation of saline and alkaline soils. In the recent years, this crop has assumed great significance due to the presence of a good quality of gum in the endosperm of its seed. On whole seed guar contain 35-40 % of endosperm which in turn contains 80 % galactomannan. The natural polysaccharide water-soluble polymer found in the endosperm, as galactomannan gum, is the chief product used in many industries . Due to diversified uses of cluster bean gum in textile, paper, explosive and mining industries, pharmaceuticals, cosmetic goods and food stuffs, it has ever increasing demand in the international market. Cluster bean seed meal a by product of guar gum industries consisting of seed coat and germ cell material is a good source of protein. It is principally used as a feed for livestock and poultry.

India is the largest producer of Guar and contributes 80 percent of total Guar production in the world. In India, Guar crop is cultivated mainly during Kharif season, with an annual production of around 2 million metric tons. Guar crop has experienced a remarkable journey from a traditional crop grown on marginal lands mainly for food, animal feed and fodder to a crop with various industrial usages. Guar gum is an important ingredient in producing food emulsifier, food additive, food thickener and other Guar gum products. The unique binding, thickening and emulsifying quality of guar gum powder obtained from guar seed has made it a much sought after product in international market.

Total production of Guar bean in India is estimated to have crossed 2.7 million metric tons during the agricultural year 2013-14 due to good weather conditions in the major Guar producing areas in India. With a moderate production of 2,50,000 metric tons Pakistan which is another important guar producing area, the total global production of Guar bean is estimated to have crossed 3 million metric tons during 2013-14. Presently, India accounts for more than three-fourth (or nearly 80 percent) of the total world Guar bean production (Figure-1). The other major producers of Guar are Pakistan, USA, South Africa, Malawi, Zaire and Sudan.

Although DNA and RNA finger printing could be accomplished by several methods, RAPD analysis is appealing because it does not require genomic sequence or radiolabelling and can distinguish variability not only in organisms but even between different strains or isolates of the same organism. RAPD markers are DNA fragments from PCR amplification of random segments of genomic DNA with single primer of arbitrary nucleotide sequence. RAPD polymorphism is detected by using oligonucleotide, usually 10 bases long of random sequence as primer in PCR reaction. In a strain that has in its genomic DNA sequence complementary to primer oligonucleotide, PCR product will be detected in gel. While those strains that do not have the complementary sequence, no product will be detected. Some of these bands are present in some and absent in other strains. These bands constitute RAPD loci. Thus RAPD generated by specific oligonucleotides are used in PCR reaction. Considering above facts, the present research plan was designed with the objective to study genetic diversity of cluster bean by using RAPD markers and cluster analysis.

II. IMPORTANCE OF RESEARCH WORK

- It is important tool for evaluating genetic diversity and determining cultivars identity (molecular fingerprinting).
- Plant breeder can succeed in their breeding program by relying on the diversity or variation in plant population.
- It also important to study phylogenetic relationship among the species and subspecies.

Development of cultivars with multiple resistances to biotic and abiotic stresses is an important breeding objective for chickpea. The analysis of genetic diversity and relatedness between individuals within a species or between different species of populations are phylogenetic studies which were initially conducted using qualitative and quantitative traits, which are mostly morphological. These analyses are mostly based on quantitative traits that are highly influenced by environmental effects and require tedious statistical procedures.

A total of Five varieties of Cluster bean (*Cyamopsis tetragonoloba* Taub.) Collected from All India Co-ordinated Vegetable Improvement Project, (AICVIP) MPKV Rahuri .

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Table.1 Cluster Bean Five Varieties

Sr. No	Variety Name
1	RHRCB 22-5
2	RHRCB 29-2
3	RHRCB 30-1
4	RHRCB 34-4
5	RHRCB 41-3

Isolation of Genomic DNA and purification

The protocol of cetyl trimethyl ammonium bromide (CTAB) DNA extraction method of Saghai-Marroof (1984).

Table.2 Primers selected for RAPD analysis of the selected varieties

Sr. No	Primer	Primer sequence
1	OPB-01	5' GTTTCGCTCC 3'
2	OPB-09	5' GTGGTCCGCA 3'
3	OPB-06	5' GGTCCCTAAC 3'
4	OPA-03	5' CATCCCCCTG 3'

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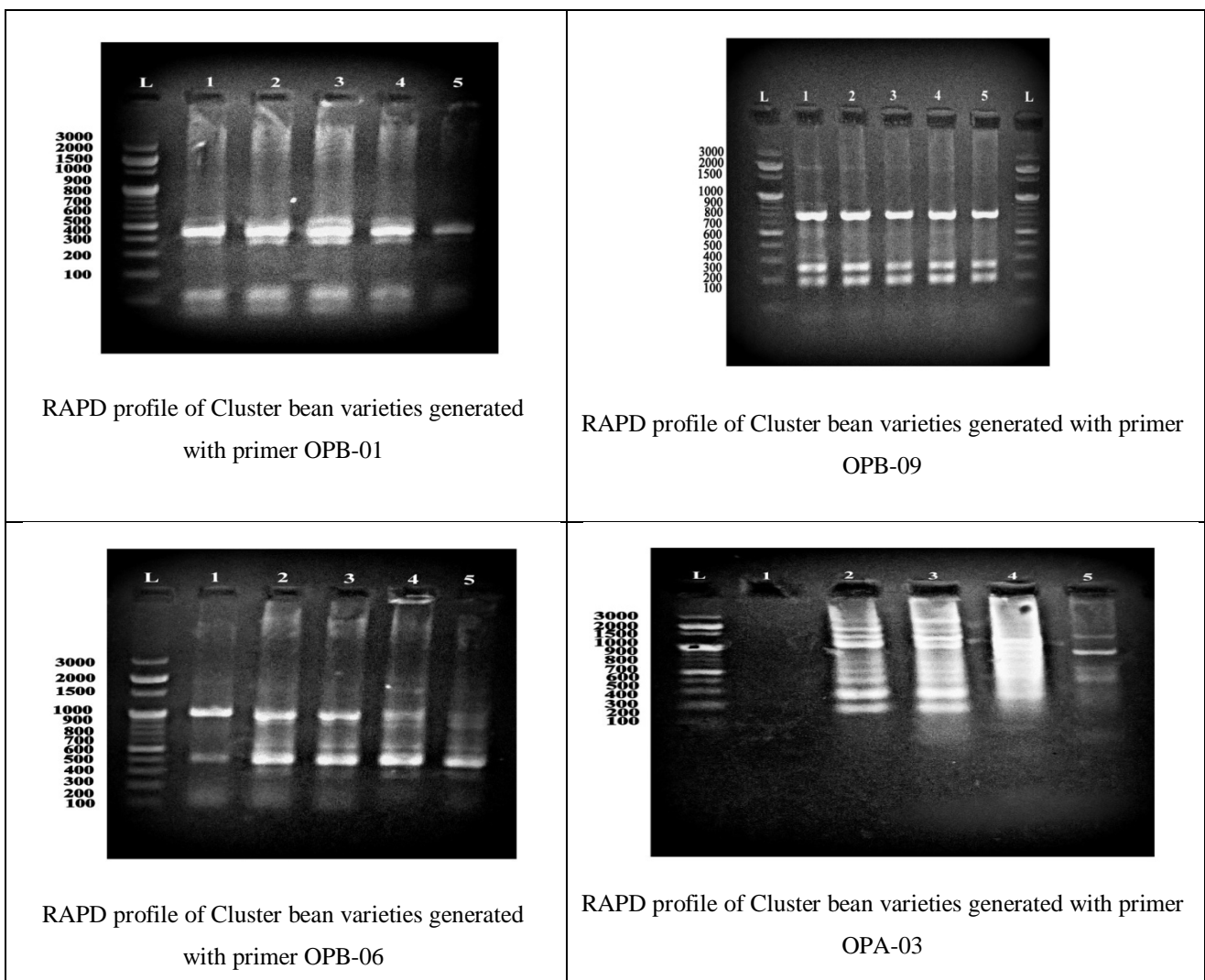
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IV.EXPERIMENTAL RESULTS

Fig.1 Banding pattern of RAPD



L- 100 bp Ladder 1.RHRCB 22-5 2.RHRCB 29-2 3.RHRCB 30-1 4.RHRCB 34-4 5.RHRCB 41-1

After isolation of genomic DNA from 5 cluster bean genotypes were subjected to PCR by using RAPD primers, Four RAPD primers were screened which successfully discriminated the *C. Tetragonoloba* species.

The amplified profiles for all the primers were compared with each other and bands of DNA fragments were scored as '1' for, presence and '0' for absence, generating '0' and '1' matrix missing for '9'.

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Table.2 List of RAPD primers, Polymorphic amplicon generated and percent polymorphism

Sr. No	Primer Code	Total No. of amplicon	No.of polymorphic amplicon	Percent Polymorphism
1	OPB-01	2	0	0
2	OPB-09	3	0	0
3	OPB-06	3	1	33.33
4	OPA-03	8	8	100
	Total	16	9	33.33

Average number of amplicon per primer = 4

Average number of polymorphic amplicon per primer = 2.5

Total percent polymorphism by RAPD marker = 33.33

A total of four primers generated 16 amplicons with an average of 4 amplicons per primer (Table 2). Of the total amplicons, 2.5 amplicons were found polymorphic. They showed 33.33 per cent polymorphism and the average number of polymorphic bands per primer were 2.5 (Table 2). Different primers produced a different level of polymorphism among the different varieties .

The primer OPA-03 was the most informative as it generated highest number of polymorphic amplicon i.e.8. The primer OPB-01 and OPB-9 stood least informative and was able to generate 0 polymorphic bands. The average size of fragments was between 100 to 3000 bp (Fig.1).

Table .3 Average Jaccard's binary similarity matrix values of each varieties obtained by RAPD marker

	RHRCB 22-5	RHRCB 29-2	RHRCB 30-1	RHRCB 34-4	RHRCB 41-3
RHRCB 22-5	1.00				
RHRCB 29-2	0.50	1.00			
RHRCB 30-1	0.44	0.85	1.00		
RHRCB 34-4	0.50	0.67	0.67	1.00	
RHRCB 41-3	0.29	0.40	0.44	0.50	1.00

Binary similarity matrix values in the range of 0.29 to 0.85 between cluster bean varieties. In the RAPD marker analysis, two cluster bean varieties RHRCB-29-2 and RHRCB-30-1 showed highest similarity matrix 0.85. (Table No.3)

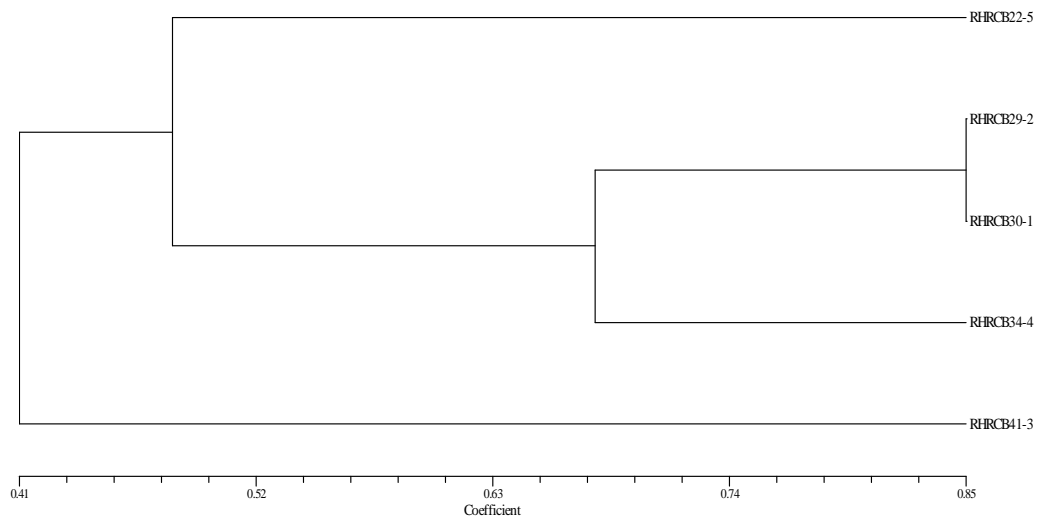
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Fig.2 Dendrogram generated by RAPD Marker



Dendrogram generated by RAPD Marker the two cluster A and cluster B had 41 % genetic similarity cluster A comprises 4 varieties where as cluster B comprising 1 varieties.

The cluster A was further divided in to 2 sub cluster named A_1 and A_2 these two cluster had similarity matrix of 0.47. Among the varieties of sub cluster A_2 the higher similarity found in between RHRCB-29-2, and RHRCB-30-1 showed 0.85 similarity. The another sub cluster A_1 comprising one cluster bean varieties that is RHRCB-22-5. Cluster B which comprised of 1 varieties that is RHRCB-41-3.

V.DISCUSSION

Pabal *et al.*(2008) studied that good quality of genomic DNA was isolated from 15 independent cluster bean cultivars and conditions were optimized for further amplification by PCR. An evaluation was made for the amplification of RAPD as a Dendrogram was prepared. Cluster bean cultivars revealed significant polyimorphism with reference RAPD markers showing authentic genotypic diversity among its races. Out of total 17 operon primers employed; 3 OPQ primers showed 19 amplicons in the size range of 100 to 6000 bp, out of these 15 amplicons were polymorphic with 78.94% polymorphism. Dendrogram obtained by RAPD marker and NTSYS PC using Jaccard's similarity coefficient values in the range of 0.70 to 0.80 between cluster bean varieties.

In the present study, genetic diversity among cluster bean varieties were analyzed by 4 RAPD primers among 5 varieties, total 9 polymorphic DNA fragments were generated. The Jaccards binary similarity matrix among all cultivars ranged from 0.29-0.85. After isolation of genomic DNA from all 5 varieties of cluster bean DNA samples were subjected to PCR using RAPD primers. A total of four primers generated 16 amplicons with an average of 4 amplicons per primer. Of the total amplicons, 2.5 amplicons were found polymorphic. They showed 33.33 per cent polymorphism and the average number of polymorphic bands per primer were 2.5. Different primers produced a different level of polymorphism among the different varieties. The data obtained by RAPD markers was analyzed by NTSYS-PC and dendrogram was generated by using Jaccard's similarity coefficient.

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VI. CONCLUSIONS

- RAPD markers method effectively classify the varieties belonging to *Cluster* species.
- Grouping results from cluster analysis based on NTSYS-PC showed that, the varieties chosen had narrow genetic variation to a considerable extent.
- The varieties with distinct DNA profiles provided useful information for selection of parents to develop new cluster bean hybrids.
- RAPD primer OPA-03 generated maximum number of amplicons *i.e.* 8 and OPA-03 primer showed highest polymorphism *i.e.* 100 %.

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