RESEARCH NOTE

Molecular assessment of genetic diversity in cluster bean (*Cyamopsis tetragonoloba*) genotypes

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Introduction

Cluster bean (*Cyamopsis tetragonoloba*) is an important leguminous herb, highly adapted to arid and semi-arid parts of the world requiring low inputs and care. The seeds are highly valued for industrial gum. It is cultivated mainly in rainy season as a rainfed crop in arid zones of India and various other parts of the world (Pathak *et al*. 2009). In conventional guar breeding, usually one considers the phenotypes of the plant in selection for high yield and quality parameters. These traits have been used for the assessment of genetic diversity in cluster bean (Dwivedi *et al*. 1999; Henry and Mathur 2005) but such traits are influenced by environmental factors and developmental stage of the plant. Molecular markers offer several advantages over the conventional breeding tools for selection of diverse parents. Randomly amplified polymorphic DNA (RAPD) uses arbitrary 10-base primers to amplify the random portion of the genome (Williams *et al*. 1990). The data from RAPD analysis have indicated greater diversity than allozymes in plant species (Esselman *et al*. 1999, 2000). It is high-throughput marker technology, which allows the analysis of large number of individuals with a large number of markers in relatively short time, as only a few primers allow the generation of sufficient data to obtain a robust estimate of diversity index. RAPD markers are based on amplified arbitrary sequences and sample a wider part of the genome than ISSR primers and have allowed the resolution of complex taxonomic relationships (Cottrell *et al*. 1997; Casiva *et al*. 2002).

The present study was carried out to find the extent of genetic diversity among 32 genotypes of cluster bean collected from different geographical regions of India for crop improvement and conservation.

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Materials and methods

Seeds of 32 genotypes of *C. tetragonoloba* were procured from three major cluster bean growing states: Rajasthan, Haryana and Gujarat (table 1). Plants were raised in pots at ambient temperatures at Central Arid Zone Research Institute, Jodhpur, Rajasthan.

DNA isolation

The total DNA was extracted from approximately 100 mg of five-days-old seedlings of cluster bean, crushed with micropestle in a 1.5 mL conical micro-centrifuge tubes with liquid nitrogen. Dneasy® plant mini kit protocols (Qiagen GmbH, Hilden, Germany) were used for DNA isolation.

RAPD analysis

Multilocus genotyping by RAPD was performed using 10 decamer arbitrary primers (Operon Technologies, Alamed, USA), of which five primers generated reproducible and highly polymorphic bands. Amplification was performed in a total reaction mixture of 25 μL. Each reaction mixture containing: decamer primer, 2 μL (50 pmol μL^{-1}); dNTP mix, 2 μL (2 mM each from MBI Fermentas, Maryland, USA); $MgCl_2$, 1 μL (25 mM, MBI Fermentas, Maryland, USA); Taq DNA polymerase, 3 μL (5 U μL^{-1}, Sigma, Munich, Germany); 10× PCR buffer, 2.5 μL (100 mM Tris-HCl, pH 8.3, 15 mM $MgCl_2$, 250 mM KCl) and 14.5 μL of dH_2O, 4 μL of genomic DNA (~40–60 ng) was added to each reaction mixture. RAPD-PCR amplification were performed in a gradient thermal cycler (Corbett Research, San Francisco, USA) with lid heating option at 110°C. PCR conditions were set with an initial denaturation step of 94°C for 3 min, followed by 38 amplification cycles of 94°C for 40 s, 50°C for 40 s and 72°C for 2 min and final elongation at 72°C for 10 min.

PCR amplification products were electrophoretically separated on 1.6% agarose gel (Sigma, Munich, Germany) prepared in 1× TAE (Tris-acetic acid-EDTA). The gel was run

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for 3 h at 50 V. The staining was done with ethidium bromide and visualized under 300 nm UV light and photographed. The gel photographs were scored for presence (1) and absence (0) of scorable bands with the assumption of positional homology. To establish the genetic relationship among the genotypes, similarity coefficients were calculated between genotypes and combined dendrogram of all the five primer products was drawn using unweighted pair group method using arithmetic averages algorithm (UPGMA) of the NTSYS-pc, version 2.02h programe (Sneath and Sokal 1973; Rohlf 1997).

Results and discussion

Out of 10 random primers, five primers (OPA-16, OPP-7, OPB-12, OPP-9 and OPA-14) were selected for the analysis as others gave either indistinct, sub-optimal or monomorphic amplification products. These five selected primers generated a total of 49 amplicons of which 39 were polymorphic and exhibited high degree of marker index ranging from 66.6% to 87.5% polymorphism in banding pattern (table 2). Such degree of polymorphism in banding pattern has also been reported in *Ziziphus* (Devanshi *et al*. 2007) and *Vigna radiata* (Lavanya *et al*. 2008). The number of PCR amplified products formed ranged from 5 (OPA-14) to 16 (OPA-16) with an average of about 10 bands per primer. A representative RAPD profile generated by OPA-16 is shown in figures 1 and 2 of electronic supplementary material at http.www.ias.ac.in/jgenet/.

The UPGMA dendrogram obtained from the cluster and using Jaccard's coefficient showed two major and four minor clusters (figure 1) while RGC 1038, CAZG 6 and RGC 1002 did not fall in any cluster. Minor cluster 1 A, comprised of three genotypes RGC 1033, RGC 1055 and RGC 1077 showing their geographical relationship. Minor cluster 1 B comprised of 11 genotypes. The analysis of grouping pattern of this cluster for the genotypes did not correspond to their geographical regions. Minor clusters 2 A and 2 B included eight and seven genotypes, respectively.

The most distinct genotypes RGC 1002 and CAZG 6, exhibited the maximum genetic diversity of about 37%. The maximum genetic similarity up to 98% was observed between the genotypes RGC 2021 and CLBH 201. The RAPD markers used in the present study separated all the 32-cluster bean genotypes from each other and revealed considerable

Table 1. Source of *Cyamopsis tetragonoloba* genotypes.

Name of genotypes	No. of genotypes	Source
RGC 1088, RGC 1092, RGC 1033, RGC 1038, RGC 1078, RGC 1077, RGC 1076, RGC 2021, RGC 1059, RGC 1055, RGC 1029, RGC 1002, RGC 986	13	RAU, Durgapura, Rajasthan
HGS 365, HGS 02-29, HGS 155-156, HGS 891, HGS 02-20, HGS 02-1, HGS 870, HGS 880, HGS 884	9	CCS HAU, Haryana
GAUG 9808, GAUG 0101, GAUG 003, GAUG 004	4	GAU, Gujarat
VIKAS 35, CLBH 201	2	Miscellaneous
RGM 114, RGM 115	2	RAU, Jodhpur, Rajasthan
CAZG 6	1	CAZRI, Jodhpur, Rajasthan
AVKG 73	1	Avikanagar, Rajasthan

Table 2. Details of arbitrary primers showing polymorphic amplions generated from 32 genotypes of cluster bean.

Primers	Sequences 5′ to 3′	GC content (%)	Total number of bands	Total number of polymorphic bands	Polymorphism (%)
OPA 16	AGCCAGCGAA	60	16	14	87.5
OPP 7	GTCCATGCCA	60	9	6	66.67
OPB 12	CCTTGACGCA	60	13	11	84.61
OPP 9	GTGGTCCGCA	70	6	4	66.67
OPA 14	TCTGTGCTGG	60	5	4	80.0
	Total		49	39	79.59

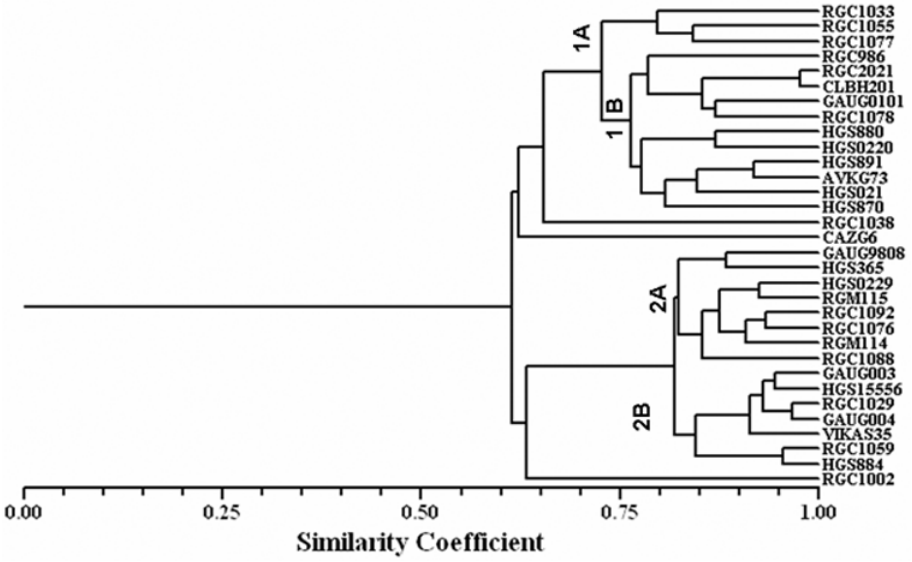

Figure 1. UPGMA dendrogram showing genetic diversity in 32 genotypes of *Cyamopsis tetragonoloba*.

genetic diversity. On the basis of similarity coefficient, the most distinct genotypes viz., RGC 1038, CAZG 6, RGC 1002, RGC 1033, RGC 986, HGS 880, HGS 870, HGS 884 and RGC 1088 can be utilized in guar improvement programmes.

The genotypes from the same geographical region were also grouped into different clusters. The different genotypes may have whole or partial common pedigrees and may have been subjected to the same selection during their breeding but are still distinguishable from each other on the basis of RAPD profiles. Bisht *et al*. (1998), Manivannan *et al*. (1998) and Lavanya *et al*. (2008) have also reported lack of correlation between geographic and genetic diversity in other legumes.

RAPD markers have been used for the identification of cultivars and the genetic relationships among cultivars of other leguminous crops including *Phaseolus vulgaris* (Skroch *et al*. 1992), *V. unguiculata* (Mignouna *et al*. 1998), *V. angularis* (Yee *et al*. 1999) and *V. radiata* (Lakanpaul *et al*. 2000; Lavanya *et al*. 2008). The present study has revealed wide genetic base in *C. tetragonoloba* genotypes for crop improvement.

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