EST-SSRs Provide a Good Measure of Genetic Diversity for Improvement of Gum Content in Cluster Bean

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Introduction

Cyamopsis tetragonoloba (L.) Taub., commonly known as *guar* is an important multipurpose arid leguminous crop of India, mainly cultivated in north-western parts of India. The pods of the guar plant grow in clusters giving guar the common name of clusterbean. It is mainly grown for feed, green fodder, vegetable and green manuring. Its seeds are also an important source of galactomannan (guar gum) which is used as a food ingredient and more recently as a neutraceutical. Guar gum is also having pharmaceutical importance and found to be effective in osteoarthritis, as artificial cervical mucus and for anticancer medicine in the treatment of colorectal cancer. Particularly in 2012, world demand for guar gum has skyrocketed and the price of guar has increased by approximately 230 per cent and even more, mainly because of increased oilfield shale gas demand. As a consequence, there has been a 75 per cent jump in exports from India, the largest guar producing country (Gresta *et al*., 2013) due to which India's much neglected and little-known galactomannan became its biggest agricultural item of export.

To fulfill all these purposes, the increasing demand of the guar seeds cannot be compensated by present resources. Therefore, new varieties with higher gum content are urgently needed. For this, knowledge of genetic diversity among the varieties has immense importance for plant breeders. Larger variability in the initial breeding material ensures better chances of producing new desired forms of a crop (Pathak *et al*., 2011). Molecular markers offer a promising tool for plant breeding efforts. SSRs are highly valued molecular markers for studying genetic diversity in crop plants. But unfortunately, clusterbean is a genomically poor crop as no genomic SSRs have been developed. Literature available on the nature and magnitude of diversity in clusterbean indicates that the studies of this kind are scanty and not properly documented. Studies were therefore, required to assess the extent of genetic variability in association with the galactomannan content using reliable EST-SSRs.

Materials and Methods

Seeds of 139 guar [*Cyamopsis tetragonoloba* (L.) Taub.] genotypes, alongwith two wild species, *i.e. C. serrata* and *C. senegelensis*, were procured from Department of Genetics and Plant Breeding, CCS Haryana Agricultural University, Hisar and National Bureau of Plant Genetic Resources (NBPGR), New Delhi. Galactomannan content in their seeds was estimated using modified method of Joshi (2004). Depending upon the galactomannan content, a total of 20 genotypes with low (<20 %) and high (>35 %) content, were selected and then raised under net house conditions.

Genomic DNA was isolated from young leaves of all the genotypes of guar by Cetyl Trimethyl Ammonium Bromide (CTAB) extraction method modified by Saghai-Maroof *et al.* (1984).

EST database, provided by Naoumkina *et al.* (2007) at National Centre for Biotechnological Information (http://www.ncbi.nlm.nih.gov/genbank/), was used to retrieve the sequences related to carbohydrate (specifically galactomannan) metabolism. Simple Sequence Repeat Identification Tool (SSRIT) software available at the GRAMENE website (http://www.gramene.org/db/markers/ssrtool), was used to find SSRs. Only those ESTs, which contain SSR, were used for primer designing using online available primer designing software, Primer3 (v. 0.4.0) (http://bioinfo.ut.ee/primer3-0.4.0/).

The sequences related to galactomannan content were identified from EST database of *Cyamopsis tetragonoloba* out of which SSR primers were designed and used for molecular diversity analysis of selected genotypes via PCR amplification and PAGE analysis. Based on the 0/1 matrix of allele scoring, genetic similarity coefficient was calculated to estimate all pairwise differences in the amplification product for all genotypes using 'SIMQUAL' sub-programme of NTSYS-pc (version 2.0) software (Numerical Taxonomy and Multivariate Analysis System Programme). Dendrogram was

constructed by using distance matrix by the Unweighted Pair-Group Method with Arithmetic Average (UPGMA) sub-programme of NTSYS-PC.

Results and Discussion

Galactomannan content from seeds of all clusterbean genotypes alongwith two wild relatives *i.e.*, *C. serrata* and *C. senegelensis* was found to be in the range of 15.12 to 36.68 per cent. The wild genotype, *C. serrata* has 18.32 per cent while highest gum content was found in HG 3-2 (36.68%). A total of 16476 EST sequences were sourced from EST database of *Cyamopsis tetragonoloba* available at NCBI public domain (Accessions EG974821 to EG991296). Sixty six sequences related to galactomannan content were selected, from which 36 EST-SSR primers were designed. The amplification of DNA samples which produced reproducible bands were considered for analysis. Each amplified product was scored across all the 20 genotypes for 36 EST-SSR primers. Band positions for each guar genotype were found to be polymorphic and were scored as either present (1) or absent (0). The scores were entered into a database program (Microsoft Excel) and compiled in a binary matrix for phonetic analysis using NTSYS-pc (Numerical Taxonomy & Multivariate Analysis) system.

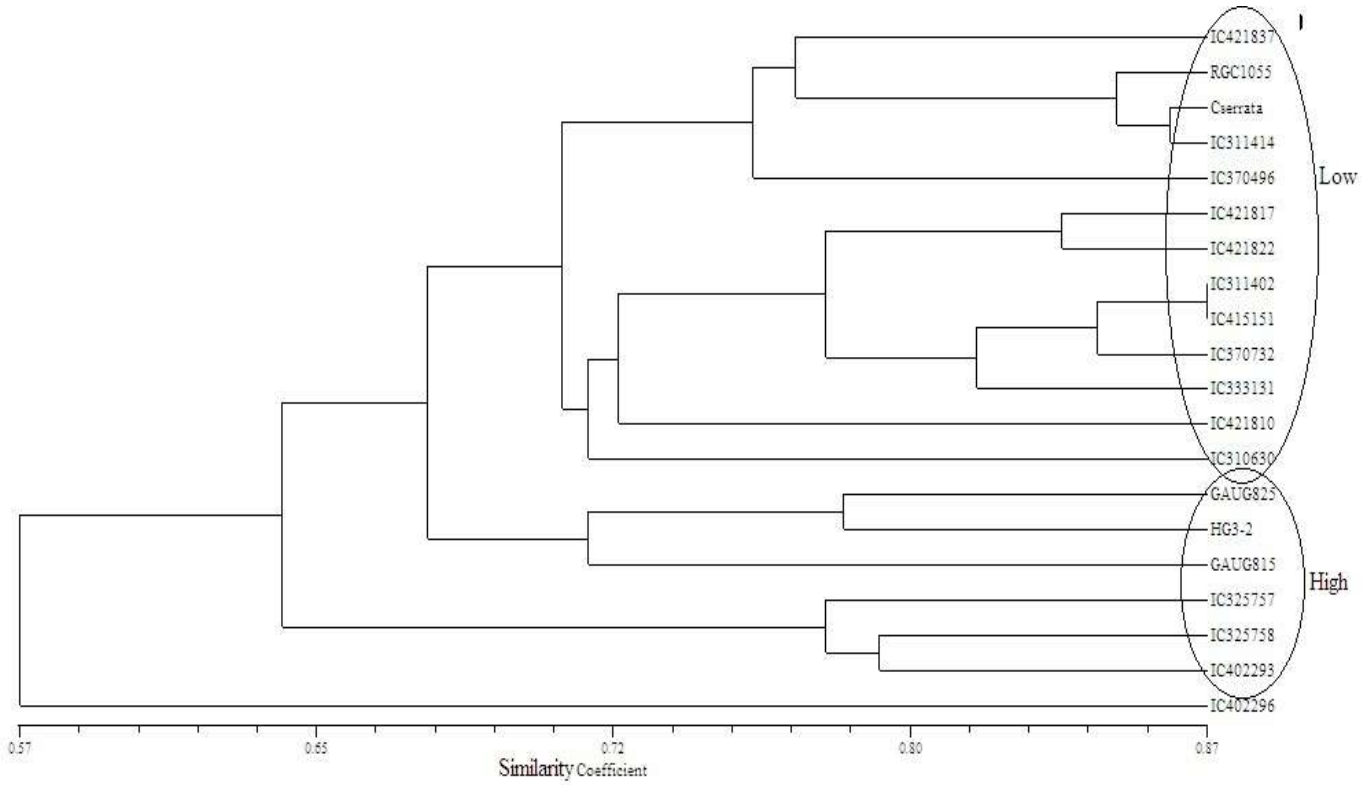

Fig. 1: Dendrogram showing genetic similarity coefficient and relatedness among clusterbean genotypes based on galactomannan content

The diversity analysis using NTSYS-PC (Fig. 1) revealed two separate groups (high and low) relevant to galactomannan content with similarity coefficient of 0.67. The high degree of polymorphism in SSRs results from different copy number of basic motifs or internal heterogeneity of the sequences. Variation in the number of tandem repeats results in PCR product variation. Microsatellites are among the fastest evolving DNA sequences, as they frequently change their length by means of addition or deletion of repeat units. This is due to the high propensity for slippage, which repeat arrays demonstrate during DNA replication. Greater manifestation of heterosis is expected in cross combinations involving the parents from the most divergent clusters.

Conclusion

Knowledge about the size and nature of diversity of the germplasm provides a platform for designing crop improvement programmes. Breeding strategies need to exploit existing variation within the clusterbean germplasm for widening the genetic base. Eventually, the results of the present study can be used for varietal/genotype identification and parental

selection, and will be helpful in augmentation of the clusterbean improvement programme. This study can be used in developing mapping population and for QTL mapping which can be used for marker assisted breeding and could be beneficial to the plant breeders, for enhancement of galactomannan content.

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