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SSR Markers for Polymorphism Detection in Released Varieties and Hybrids of Pigeonpea [*Cajanus cajan* (L.) Millspaugh] of Gujarat

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ABSTRACT

Pigeonpea (Cajanus cajan) is an important subsistence crop in India where traditional landraces and improved hybrids are grown along side each other. SSR primers used to determine varietal/hybrid specific informative molecular markers for precise identification of varieties and hybrid of pigeonpea. DNA isolation was carried out by CTAB method and quality as analysed on nanodrop, the absorbance ratio of DNA at A260/A280 and finally with electrophoresis. Eleven of the 12 SSR primers studied amplified 13 alleles. Primer CCttc001 was more useful on account of its ability to amplify maximum number of 2 alleles and were found to be the other promising SSR primers for detecting polymorphism among the different genotypes of pigeonpea. CCtta002, CCgtt001, CCtacccg001 and CCtc002 primers did not amplified genomic DNA of pigeonpea. SSR primers classified the eleven genotypes of pigeonpea into two different clusters with lot of variation in number and inclusion of genotypes in sub groups. Though these techniques indicated genotype GT-100 to be distantly apart from genotype GT 288 A, yet comparing other set of genotypes, GT-101 were distantly apart in SSR analysis. This indicated no uniformity in grouping of genotypes based on SSR primers.

Key words: *DNA, SSR primers, Genotypes, Molecular Markers*

INTRODUCTION

Pulses occupy an important place in Indian agriculture. Within this protein-rich group of crops, red gram or pigeonpea [*Cajanus cajan* (L.) Millsp.] occupies an important place among rainfed resource poor farmers because it provides quality food, fuel, wood, and fodder. Its soil rejuvenation qualities such as release of soil-bound phosphorous, fixation of atmospheric nitrogen, recycling of soil nutrients, and addition of organic matter and other nutrients make pigeonpea an ideal crop of sustainable agriculture in the tropical and sub-tropical regions of India. Although pigeonpea is globally grown on 5.2 m ha land in about 50 countries, its 77% area is in India (FAO, 2008). In Gujarat early maturing varieties are preferred though mid late maturing varieties are also liked in Suarashtra and Central Gujarat.

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A CGMS based hybrid, GTH-1 has been released for cultivation in Gujarat. Stable varieties of pigeonpea like GT 100, GT 101, GT 102, GT 1, Banas, BSMR 853 (Vaishali), SKNP 505 and AGT 2 have been recommended for cultivation in Gujarat. The Simple Sequence Repeats (SSR) markers are very valuable molecular tools as they are co-dominant, easy and economically assayed by PCR and can detect high levels of allelic diversity. They are considered ideal for DNA fingerprinting and varietal identification particularly the hybrids because of their ability to detect large number of discrete alleles repeatedly, accurately and efficiently[6] conducted a study to develop microsatellite markers and evaluate their potential for use in pigeonpea genetics and breeding. However, SSR have been found to be less informative in pigeonpea[9].

MATERIALS AND METHODS

The leaf materials were collected for analysis from pigeonpea plants of each genotype. Total genomic DNA was extracted from the leaves of 9 days seedlings by Cetyl trimethyl ammonium bromide (CTAB) method[3] with some modifications. The DNA was quantified using 0.8 % agarose gel.

SSR marker assay:

The SSRs analysis was done following the procedure given by Odeny *et al*[7]., with minor modifications. In all 12 microsatellite marker were used that were obtained from Bangalore GeNei, Bangalore. PCRs was performed in a 25 µl reaction volume with 2.50 µl of 10X PCR buffer, 2 µl of 10 mM dNTPs, 1 µl of 10 pmol/ µl forward primer, 1 µl of 10 pmol/ µl reverse primer, 0.5 U of *Taq* polymerase (Genei, Bangalore, India) and 2 µl (50 ng) of template DNA; in 96-well microtiter plate using mastercycler (Bio-Rad, USA). A touchdown PCR programme was used to amplify the DNA fragments: the initial denaturation was for 5 min at 95^0C. This was followed by initial 30 cycles of denaturation for 40 s at 94^0C, annealing for 1 min. at 55-60 ^{0}C and extension for 2 min. at 72^0C. Subsequently 30 cycles of denaturation at 94^0C for 40 s, annealing for 1 min at 55-60^0C and extension for 2 min at 72^0C followed by 5 min of final extension at 72^0C and stored at 4^0C. PCR products were then subjected to electrophoresis with 100 bp Marker DNA in 2.5 per cent agarose gel at voltage of 6V/cm using 1X TBE buffer and Ethidium Bromide staining (0.5 µg/ml). On completion of run, gel was viewed under UV light and photographed using Upland and Gene Genius Bio System, SynGene, UK.

Table 1.1: Size, number of amplified bands, per cent polymorphism and PIC obtained by SSR primers

S.No.	SSR No.	Total Band	Monomorphic Band	Polymorphic Band	Shared Band	% P	PIC value
1	CCtta001	1	1	0	0	0	0
	CCtta001						
2	CCtta003	1	1	0	0	0	0
	CCtta003						
3	CCtta004	1	0	1	0	1	0
	CCtta004						
4	CCttc001	2	1	1	1	100	0.337
	CCttc001						
5	CCac001	1	1	0	0	0	0
	CCac001						
6	CCat001	1	1	0	0	0	0
	CCat001						
7	CCat002	1	0	1	0	1	0
	CCat002						
8	CCat003	1	1	0	0	0	0
	CCat003						
9	CCat004	1	1	0	0	0	0
	CCat004						
10	CCcat001	1	1	0	0	0	0
	CCcat001						
11	CCggt001	1	0	1	0	1	0
	CCggt001						
12	CCtacccg001	1	0	1	0	1	0
	CCtacccg001						
	Total	**13**	**8**	**5**	**1**	-	-
	Mean	**1.08**	**0.66**	**0.41**	**0.08**	**8.33**	**0.028**

SSR data scoring and analysis:

Amplified products were scored as dominant marker as '1' (for presence of band) and '0' (for absence of band) at each of the SSR loci. Pair-wise similarity coefficients were estimated using Jaccard's index[5] based on binary scores. The pair-wise similarity estimates were then used to generate a dendrogram using the NTSYS pc version 2.0 software[8]. Polymorphic information content (PIC) of each SSR primer was computed[1]. PIC estimates indicate the power of a marker for detecting polymorphism among the genotypes, depending on the number of detectable alleles ('j') and the distribution of their frequency. The PIC of SSR markers was estimated as

$$PIC_i = 1 - \sum_{j=1} P_{ij}^2$$

Where, P_{ij} is the frequency of $j^{th}$the allele for marker i, and summation extends over n alleles.

RESULTS AND DISCUSSION

Simple Sequence Repeats (SSR) Study

Sixteen microsatellite (SSR) markers were used to analyze the genetic diversity among the 11 pigeonpea genotypes. Only five of the twelve primers exhibited polymorphism. The details of amplification products are given in (Table 1.1).

Pooled SSR Results

SSR primers produced different numbers of DNA fragments, depending upon their simple sequence repeat motifs. The 12 SSR primers produced 13 bands across eleven genotypes, of which 5 were polymorphic. Among the 12 markers, maximum amplified allele size of 1450 bp and minimum amplified allele size of 143 bp. Average number of bands per primer was 1.08. PIC values varied from 0.00 to 0.33 with an average of 0.02 (Table 1.1). The SSR bands were scored for presence (1) or absence (0) among the genotypes and used for UPGMA analysis. Jaccard's similarity coefficient ranged from 0.615 to 0.923. Dendrogram generated by pooled SSR molecular data gave two main clusters, clusters A and B. Cluster A include GT 288 A and B was divided in two sub cluster B1 and B2 included GT 102, GTH 1, GT 1 and GT 100, GT 101, GTR 11, AGT 2, BANAS, BSMR 853, SKNP 505 respectively.

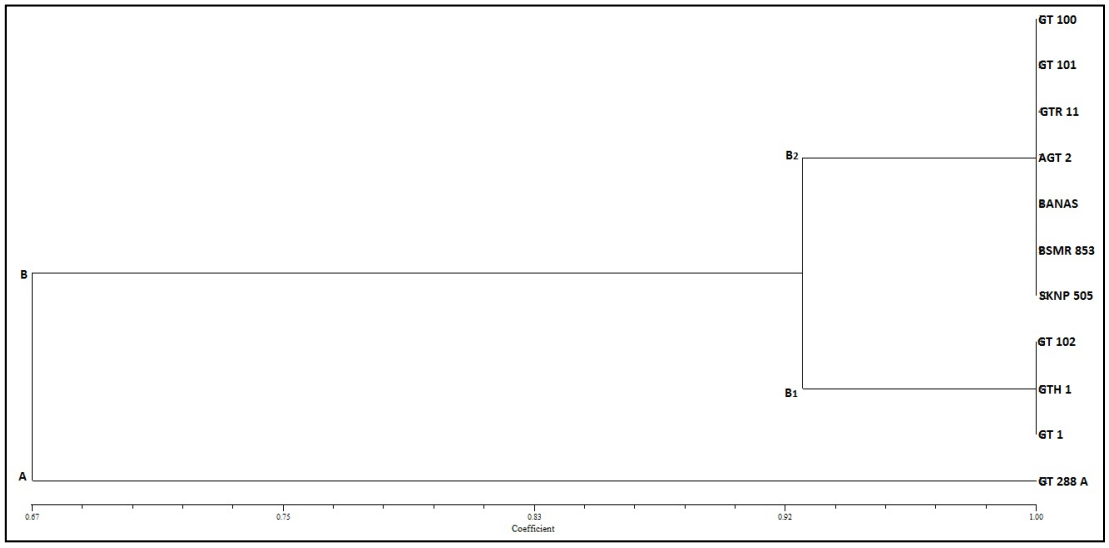

Fig.1.1: Dendrogram depicting the genetic relationship among 11 cluster bean genotypes based on the SSR data

Again pooled SSR revealed that GT-100 and CMS GT 288 A showed maximum variability compared to other nine genotypes. As microsatellite or SSR markers are highly polymorphic, reproducible, co-dominant in nature and distributed throughout the genome, they have become the ideal marker system for genetic analysis and breeding applications[4]. In

case of pigeonpea, however, only 30 SSR markers have been reported in literature[2,6]. The present study reports a new set of novel SSRs to the existing repertoire of molecular markers in pigeonpea.

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