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Validation of CAAT Box-Derived Polymorphism (CBDP) Primers for *Psidium* species and its Genotypes

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Abstract The availability of a simple, reproducible and cost effective molecular marker is a prerequisite for plant genetic analysis. CAAT box-derived polymorphism (CBDP) a novel promoter-targeted marker having a specific nucleotide sequence CAAT. It is like a RAPD because single primer is used for generating the markers. It exhibit more repodubility than RAPD markers. CBDP primers generated polymorphism in representative sets of cotton (*Gossypium* species) and linseed (*Linum usitatissimum*) cultivars. CBDP generated polymorphism only recorded in limited number of field crop. Applicability of these primer for assessment of diversity is still nil among horticultural crop specially in perennial crops. Total 25 CBDP primer is design, out of 25, only 10 primer are used for assessment of 33 genotypes including six species and 28 varieties/genotypes of *Psidium guajava*. Out of 10 only 3 primers shows polymorphism. Primers clearly indicated the current populations are diverse but reproducibility of CBDP primer is quite low for further analysis.

Keywords CBDP, RAPD, Reproducibility, Genotypes, Diversity.

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Introduction

Guava (*Psidium guajava* L.) belongs to the family Myrtaceae and is a native of tropical America. It is the fifth most important fruit crop of India occupying 4.1% of the total area under fruit cultivation. It is popular due to its year round availability, rich nutritional and medicinal value, affordable price, suitability for transportation. In India, the area under guava is estimated to be around 2.75 lakh hectares with an estimated annual production of 37.95 lakh tonnes. The major guava producing states of India are Madhya Pradesh, Maharashtra, Uttar Pradesh, Bihar, West Bengal, Andhra Pradesh, Punjab, Gujarat, Chhattisgarh and Karnataka. Guava is an excellent

source of vitamin-C, pectin and minerals in general and fruits with red and pulp are excellent source of antioxidants. Guava is also a very hardy fruit crop which can be grown on a wide range of edaphic and climatic conditions. Despite the economic and health advantages, several problems affect the productivity and quality of guava. Guava improvement breeding programs in India has been limited to the introduction and selection of genotypes with desirable agronomic characteristic. Research work on genetic diversity and characterization of wild species and local cultivars which play significant role for further crop improvement is still lacking. Molecular markers are an indispensable tool for genetic analysis of plants. Still now lot of dominant and co dominant marker was utilized for characterising the guava genotypes. In this regard CAAT box-derived polymorphism (CBDP) primers are dominant type marker which are used only on diversity analysis of field crop. In this type of marker the central CAAT nucleotides sequence of CAAT box are conserved and C is the most commonly found nucleotide prior to CAAT nucleotides. This technique is similar to RAPD because a single primer is used to study variation in organisms. However, CBDP has two main advantages over RAPD i.e., (i) markers are derived from variations present in the generich region of plant genomes, and (ii) they exhibit a higher reproducibility than RAPD markers. Based on this advantages this primer is utilizing in diversity analysis of *Psidium* species and its genotypes [5].

Table 1. List of *Psidium* species and genotypes used in the present study.

Sl. No.	Species	Varieties/genotypes
1	*Psidium guajava*	Allahabad Safeda
		Arka Kiran
		Arka Amulya
		Behat Coconut
		Black guava
		Hafsi Red
		Hissar safeda
		Hissar Surkha
		Hissar Surkha Variant
		Lalit
		Lucknow-49
		Kashipur
		Pusa Srijan
		Pant Prabhat
		Punjab Pink
		Punjab Red
		Red Peeled
		Red Type
		Yellow Type
		Sasni Collection
		Sasri Collection
		Snow White
		Sour type
		Shweta
		Thai guava
		Thai Variant 1
		Thai Variant 2
		Tamilnadu Collection
2	*Psidium friedrichsthalianum*	
3	*P. pumilum*	
4	*P. guienensis*	
5	*P. quadrangularis*	
6	*P. cattleianum*	

Materials and Methods

Plant material

Guava species and genotypes

Total 33 genotypes including six species and 28 varieties/genotypes of *Psidium guajava* conserved at Division of Fruits and Horticultural Technology, ICAR-IARI, New Delhi, were used for validation of CAAT box-derived polymorphism (CBDP) primers. The details of these genotype are given in Table 1.

DNA isolation

Young and healthy leaves were collected for isolation of DNA by using modified CTAB method and diluted to a final concentration of 10 ng/µl using TE (10 mM Tris-HCL and 1 mM EDTA) buffer.

PCR amplification using CBDP primers

The PCR was optimized by varying the concentrations of template DNA, Taq DNA polymerase, Mg^{2+} and annealing temperature. The optimized PCR mixture consisted of 50 ng template DNA, 1X PCR buffer [fermentas, Life Sciences, USA 10 mM Tris-HCl (pH. 9), 250 mM KCl 1.5mM $MgCl_2$ and 0.1% gelatin], 200 mM of each dNTPs, 0.5 µM CBDP primer and 1 U of Taq DNA polymerase (fermentas,

Table 2. List of CDBP primers used for guava characterization.

	Primer	Sequence
1	CAAT-1	5’TGA GCA CGA TCC AAT AGC 3’
2	CAAT-2	5’ TGA GCA CGA TCC AAT AAT 3’
3	CAAT-3	5’TGA GCA CGA TCC AAT AAC 3’
4	CAAT-4	5’TGA GCA CGA TCC AAT AAG 3’
5	CAAT-12	5’ TGA GCA CGA TCC AAT ATA 3’
6	CAAT-13	5’TGA GCA CGA TCC AAT GAG 3’
7	CAAT-14	5’TGA GCA CGA TCC AAT GCG 3’
8	CAAT-15	5’ TGA GCA CGA TCC AAT TGA 3’
9	CAAT-16	5’TGA GCA CGA TCC AAT TCA 3’
10	CAAT-17	5’TGA GCA CGA TCC AAT TTG 3’

Life Sciences, USA) in a total volume of 25.0 µl. The touch down PCR was performed in a thermocycler (Model G Storm, UK) following the program in which the first five cycles were run at 94°C for 1 min, 35°C for 1 min, and 72°C for 1 min, for denaturing annealing and extension, respectively. The annealing temperature was subsequently raised to 50 ^{o}C for another 35 cycles with a final extension for 10 min at 72°C . The list CBDP primers listed in Table 2.

Resolution of CBDP amplified products

The PCR-amplified products of CBDP were separated on a 2% agarose gel prepared by mixing Metaphor (Cambrex Corporation, USA) and normal agarose in the ratio of 1 : 1 using 1X TBE buffer, stained with ethidium bromide and photographed under ultraviolet light in Bio lmaging System (Alpha Imager®, USA). The gel was run in 1X TBE buffer. A 1kb DNA ladder (Fermentas, Life sciences, USA) was run alongside the amplified products to determine their approximate band size. Gel pictures were recorded under UV light gel documentation system (Alpha Imager®,USA), which are shown in Figure 1.

Results and Discussion

CBDP marker is similar to RAPD due to its applicability in studying variation in multiple loci by using single primer. However, CBDP has two main advantages over RAPD i.e., markers are derived from variations present in the gene-rich region of plant genomes and they exhibit higher reproducibility. Till now CBDP primers used to generate polymorphism in only field crop (*Gossypium* species) and linseed (*Linum usitatissimum*) cultivars. Based on their transferability and reproducibility over RAPD, an effort was made to find out the applicability of CBDP marker in genetic diversity analysis of guava germplasm. A total of 10 CBDP primers were used to characterize the 33 guava genotype. Out of 10 primers three primers were reproducible. It was found that the number of primer reproducibility is quite low for further analysis.

Conclusion

For the first time effort was made to characterize the guava germplasm using CAAT Box Derived Polymorphism. However, the primer reproductibility was quite low for further analysis.

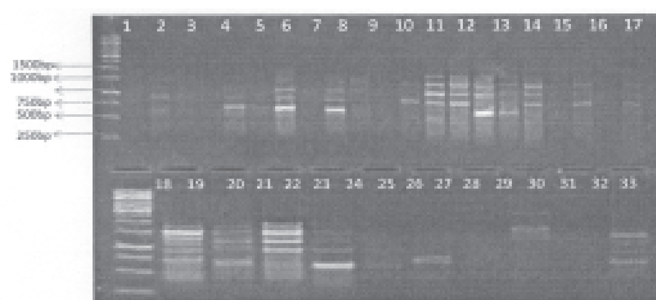

Fig. 1. CBDP amplification pattern of 33 guava genotypes.

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