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Short Communication

Molecular characterization of bacterial leaf blight and galactomannan content using RAPD markers in clusterbean (*Cyamopsis tetragonoloba* (L.) Taub)

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Clusterbean is a unique legume on account of its large spherical-shaped endosperm that contains significant amounts of galactomannan which has wide range of food and industrial applications [1, 2]. Although it is traditionally cultivated under poorly endowed situations yet it has high scope for improvement to boost industrial value and export. Among the various yield limiting factors, bacterial leaf blight (*Xanthomonas campestris* pv. *cyamopsidis*) is a serious constraint particularly during the years of prolonged and late summer rains [1]. The selection for bacterial leaf blight is influenced by environmental factors as also the stage of growth [3, 4]. Therefore, breeding resistant and susceptible genotypes through conventional and specific authenticated molecular methods can alleviate this problem. Molecular characterization of resistance and susceptible can facilitate effective and precise selection for bacterial leaf blight [5-8].

Experimental material comprised 16 genotypes of clusterbean. Amongst these, six genotypes were resistant to bacterial leaf blight and six were susceptible (Table 1). Four genotype were taken for high galactmannon content. The materials were obtained from the Centre of Excellence for Research on Pulses, Sardarkrushinagar Dantiwada Agricultural University Sardarkrushinagar, Gujarat, India. The plant genomic DNA was extracted as per modified CTAB (Cetyltrimethylethyl Ammonium Bromide) method [9, 10]. The quality and concentration of extracted DNA was ascertained using Nanodrop spectrophotometer. One hundred and sixty RAPD primers were used for the study. The volume of the reaction mixture was 20 µl that comprised 1 µl (50 ng) DNA sample, 1 µl of diluted primers (10 pmols/µl), 2 µl 10X *Taq* Buffer B, 1.2 µl dNTP mix (10 mM), 1.5 µl $Mgcl_2$ (25 mM), 1 µl Red *Taq* DNA Polymerase (1u/µl) and 12.3 µl Protease and Nuclease free water. The temperature profile used for PCR amplification comprised 94°C for 5 min, followed by 40 cycles of 94°C for 1 min, 37°C for 1 min, 72°C for 2 min and ending up with 5 min at 72°C for the final extension held at 4°C. PCR products were used for electrophoresis on 1.5 % agarose gels stained with Ethidium Bromide at 100-120 v/cm for 2.5-3.0 hrs and UV trans-illuminated gels were photographed with gel documentation system.

Forty RAPD primers were analyzed for identification of the resistance and susceptibility specific locus to bacterial leaf blight in clusterbean and 120 RAPD primers were used for identification of high and low galactomannon specific locus. The results are presented in Table 3 and Figs. 1 to 3. There were three primers *viz*., OPA 4, OPA 5 and OPA 10 that produced polymorphic bands specific to BLB susceptible and resistant locus specific genotypes. Two of them namely, OPA 5 and OPA 10 primers produced precise, distinct and unique bands that were confined only in susceptible genotypes to BLB (Figs. 1&2). OPA 5 primer produced distinct band of size 242 bp while OPA 10 produced two *suigeneris* bands of size 520 bp and 1874 bp. Contrary to this, OPA 4 produced unique band of 420 bp size that was specific only to resistant locus for BLB (Fig. 3). The results obtained in the present study needs

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Table 1. Details of genotypes of clusterbean for molecular characterization bacterial leaf blight (BLB)

S.No.	Genotypes BLB	Reaction to	Pedigree
1.	IC-102828	Susceptible	
2.	IC-103019	Susceptible	
3.	IC-102853	Susceptible	
4.	PNB	Susceptible	Pusa Mausami x Pusa Sadabahar
5.	IC-103020	Susceptible	
6.	HVG-2-30	Susceptible	Pusa Sadabahar x HGS-296
7.	HG-2-4	Resistant	Not Available
8.	HG-563	Resistant	Durga Jai x Hisar local
9.	HG-2-20	Resistant	HG-365 × SF-277
10.	RGC-471	Resistant	Local selection of Rajasthan
11.	PRT-15	Resistant	Not Available
12.	HG-365	Resistant	Durgajay x Hisar local

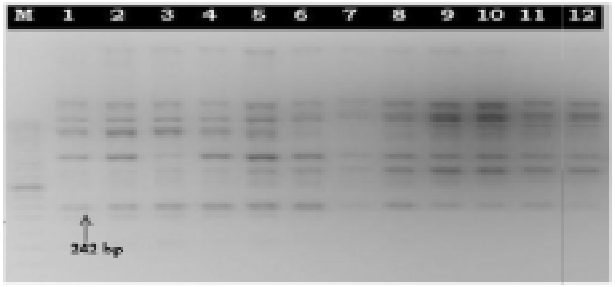

Fig. 1. The specific band of 242bp obtained in susceptible genotypes only by using RAPD primer OPA-5

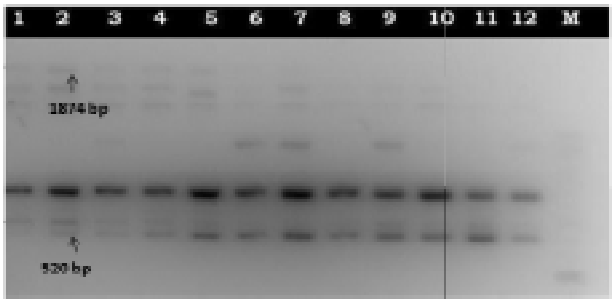

Fig. 2. The specific band of 520 and 1874 bp obtained in susceptible genotypes only by using RAPD primer OPA-10

Table 2. Details of polymorphisms revealed by three informative RAPD markers for bacterial leaf blight

S.No.	Primer	Range of amplified products (bp)	Percent polymorphism	Size of informative band (bp)	Specific to
1	OPA-4	152 to 1451	60	420	Resistance
2	OPA-5	242 to 3411	50	242	Susceptibility
3	OPA-10	445 to 3438	75	520 and 1874	susceptibility

further authentication of the linkage of the specific primers with susceptibility or resistance specific locus in segregating populations.

Four genotypes comprising two each for extremely high (IC 116603 and Gandhinagar 18) and low (GG-2 and IC 116566) galactomannan contents were used for identification of molecular marker linked to galactomannon contents. One hundred and twenty primers were utilized for identification high and low galactomannon specific locus. Six primers *viz*., OPB 12, OPI 8, OPC 2, OPI 9 OPB 14 and OPC 16 produced polymorphic bands which were specific to high and low galactomannan contents. Out of these, three primers *viz*., OPB12, OPI8 and OPC2 primers produced precise and distinct bands of size 800 bp, 350 bp and 400 bp, respectively, that were uniquely confined only to genotypes having high galactomannan contents (Fig. 4 and Table 3). Two of the other three primers *viz*., OPI

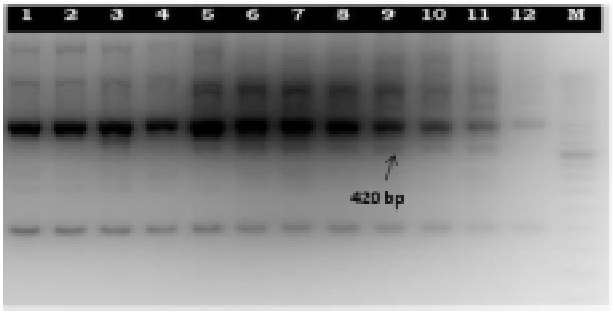

Fig. 3. The specific band of 420 bp obtained in resistant genotypes only by using RAPD primer OPA-4

9 and OPB 14 primer (Fig. 4) produced distinct band of size 500 bp and 150 bp while OPC 16 produced two *suigeneris* bands of size 600 bp and 100 bp that were precisely specific to low galactomannon contents.

As these primers specifically characterizing resistance and susceptibility specific locus to bacterial leaf blight or the high and low galactomannon content specific locus in clusterbean in the selected genotypes, the results need further confirmation in segregating

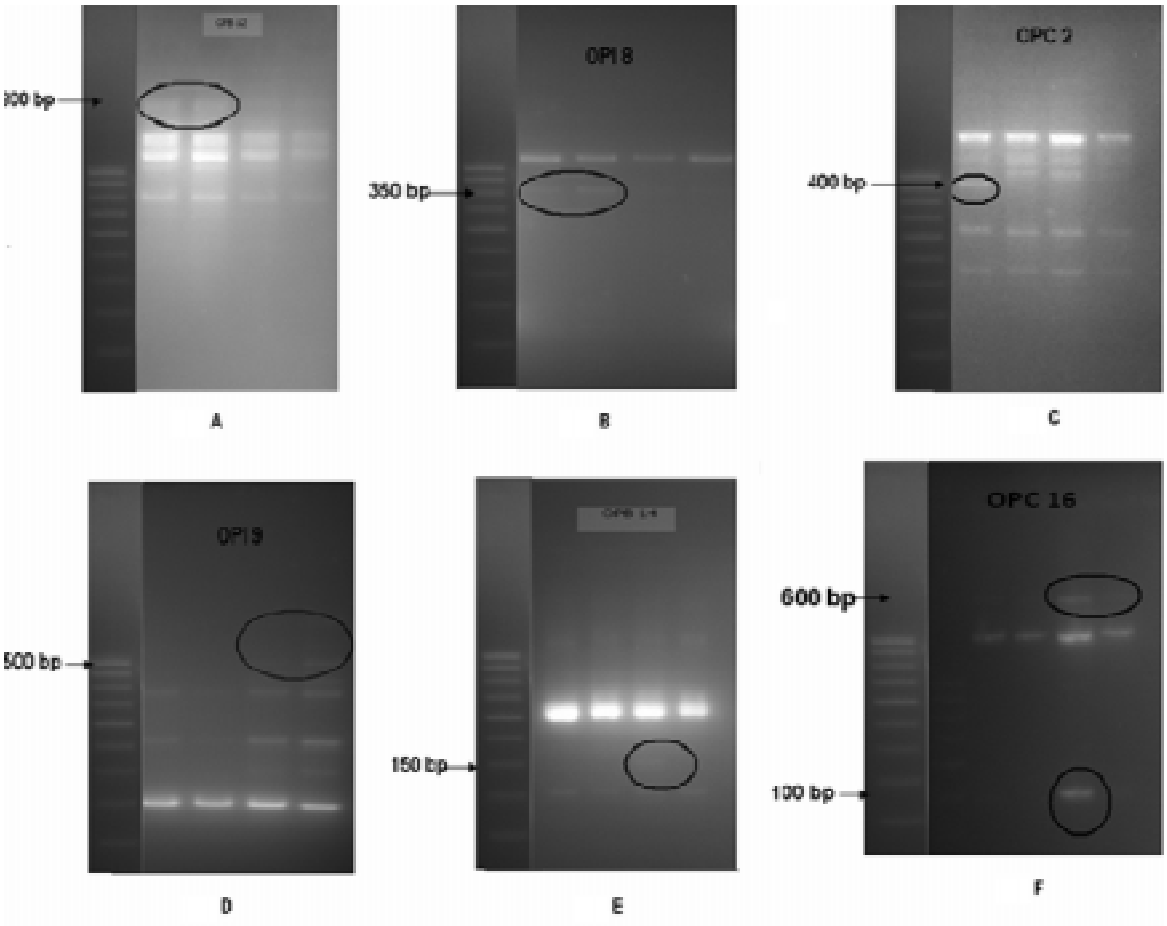

Fig. 4. Low and high galactomannan specific informative bands as revealed by 6 RAPD markers (A) OPB 12, (B)OPI 8, (C)OPC 2, (D) OPI 9, (E)OPB 14 & (F)OPC 16 in clusterbean

Table 3. Details of the polymorphic RAPD primers evincing specific informative bands in high and low galactomannan contents in clusterbean

S.No.	Primer	Size of informative band (bp)	Specific to the genotypes having galactomannon
1	OPB 12	800	High
2	OPI 8	350	High
3	OPC 2	400	High
4	OPI 9	500	Low
5	OPB 14	150	Low
6	OPC 16	600, 100	Low

populations. Superior lines of culsterbean have been earlier identified using molecular technology [11]. Further, RAPD primers being random, once the linkage of these markers with resistance and susceptibility specific locus to bacterial leaf blight or high and low galactomannon content is confirmed these RAPD markers can be converted into sequence based more precise SCAR markers.

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