Development of a dominant SCAR marker associated with an allele of *SV1* gene for sterility mosaic disease resistance in pigeonpea [*Cajanus cajan* (L.) Millsp]

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Abstract

Pigeonpea sterility mosaic disease (PSMD) caused by pigeonpea sterility mosaic virus (PPSMV) is one of the major biotic concerns that leads to serious yield losses and, hence, poses a big challenge for pigeonpea production in the Indian subcontinent. In this study the inheritance, identification and development of molecular markers associated with Bidar isolate of PPSMV resistance gene by using F_2 population was attempted. We reveal that PSMD is controlled by two genes, named as *SV1* (inhibitory) and *SV2* (resistant). Of the 300 RAPD primers, 32 primers recorded polymorphism in the parents, were screened using bulked segregant analysis method. Two markers ($IABTPPN18_{827}$ and $IABTPPAK19_{1295}$) amplified susceptible parent specific amplicon and were associated with the PSMD responsive gene at a distance of about 12.5 cM and 10 cM respectively. We developed SCAR marker associated with the *SV1* gene. Based on the sequencing data of the $IABTPPN18_{827}$ marker, we successfully developed a dominant SCAR marker, $FlntN18_{827}$, which was associated with *SV1* gene at the distance of 12.5 cM. This *SV1*-SCAR marker will be a valuable tool for marker-assisted breeding in developing PSMD resistant pigeonpea.

Key words: PIGEONPEA, PSMD, PPSMV, *SV1*, RAPD, SCAR.

Introduction

Pigeonpea (*Cajanus cajan* (L.) Millsp.) is an important food legume of the tropical and subtropical regions of Asia and Africa and it is an important protein source millions of resource-poor farmers.It is also popular as seed vegetable, grown in the kitchen garden and backyards. India is the largest producer of pigeonpea (3.29 mt) followed by Myanmar (0.57 mt) and Malawi (0.30 mt) (FAOSTAT 2014; http://faostat.fao.org), where three disease *viz*., pigeonpea sterility mosaic disease (PSMD), fusarium wilt and phytopthora blight are a serious challenge for sustainable pigeonpea production. The PSMD caused by pigeonpea sterility mosaic virus (PPSMV) is transmitted by an eriophyid mite (*Aceria cajani* Channabasavanna) and a *tenui*-like virus of asymmetric morphology as causal agent of this disease (Kumar et al. 2000). However, recent deep nucleic acid sequencing efforts on PPSMV point at its similarity to emaravirus (Elbeaino et al. 2014). Long life cycle and out crossing nature of the crop render conventional breeding efforts to be seldom successful in developing PSMD resistant pigeonpea. Multi-allelic control, linkage drag, and need for accurate phenotyping of disease have been challenging to the breeders. Although in different crosses, single genes control (Ganpathy et al. 2009; Murugesan et al. 1997; Srinivas et al. 1997) and oligo-gene nature (Gnanesh et al. 2011; Nagaraj et al. 2004; Sharma et al. 1984) and involvement of QTLs (Gnanesh et al. 2011) have been reported. Recently, Daspute et al. (2014) also reported involvement of two gene, *SV1* and *SV2* interaction governing resistance to Bidar isolate of PPSMV in ICP 8863 × BSMR 736 cross. The use of genetically linked molecular markers is expected to facilitate large-scale germplasm screening and marker assisted selection (MAS) to identify the resistance germplasm lines and development of PSMD resistant varieties / hybrids, respectively. In the present study, we report a dominant SCAR marker (*SV1*-FlnthN18$_{827}$), genetically associated with a gene (*SV1*) that confers resistance to PPSMV infection in an inhibitory manner together with (*SV2*) gene in BSMR 736 × Gullyal white cross.

Materials and methods

A. Plant material

A total of 325 F_2 population was developed from the cross BSMR 736 (resistant genotype) × Gullyal white (susceptible genotype) and F_1 seeds were obtained from Agricultural Research Station (ARS), Gulbarga,

India. The part of F_1 seeds were used for PSMD screening at ARS, Gulbarga, India during rainy season of 2008. The F2 populations along with parents were grown in the field covered with nylon net (0.5 mm size) cages to prevent insect entry and possible cross pollination, at Main Agricultural Research Station (MARS), Dharwad, India in June 2008. The matured seeds from individual F_2 plants were collected to constitute $F_{2:3}$ families and the same were used for the field evaluation in PSMD hot spot of Bidar isolate at ARS, Bidar, India in June 2009. 'BSMR736' is known to be highly resistant to PPSMV, indeterminate, semi spreading and late maturity. Similarly, 'Gullyal white' is a highly preferred for its *dhal* quality and susceptible for PPSMV infection.

B. Disease evaluation

Conformation of resistant and susceptible nature of parental lines to PPSMV at ARS, Gulbarga was done during *kharif* 2005, 2006 and 2007 following the leaf stapling technique at 2-3 leaf stage (Nene and Reddy, 1977). The reaction of F_1 plants to the PSMD was assessed by following the leaf stapling technique.. The PSMD reaction were scored at 15 days interval up to 75 days from sowing and classified as susceptible or resistant based on the PSMD symptoms. The disease reaction of the parents and $F_{2:3}$ families were assessed in the PSMD sick plot for pigeonpea at ARS, Bidar, India. The individual families were sown in two rows each with 10-15 plants per rows (in a row of 2 m length). The individual $F_{2:3}$ families were sown contiguously with ICP 8863 as check in regular intervals of every five row as spreader of PPSMV. Manifestation of PSMD chiefly depends on the availability of mite populations (Singh et al., 1999). The mite populations are usually positively correlated with rainfall, relative humidity and lower temperature. The recommended package of practices were followed to raise the crop in such a way that the manifestation of PSMD is not affected. The experimental plot chosen was positioned to have one side sugarcane field and another side the paddy crop. Both crops alongside to the pigeonpea experimental field are known to create favourable climatic condition for the built-up of mite populations and manifestation of PSMD. The artificial inoculations of PPSMV Bidar isolate were done according to "leaf stapling" and "infector-hedge" techniques in the field (Nene and Reddy 1976). Approximately >10 mite /leaf stapled is effective, heavily infected leaves from source plants were used for inoculation. Individual 325 $F_{2:3}$ families and the parents were scored at 15 days interval up to 75 days before and after ratooning for PSMD incidence during 2009 and 2010. The $F_{2:3}$ families were classified as resistant, moderately resistant and susceptible based on the percentage of disease incidence and further grouped based on the following standard scale as 0-10 %of plants infected–

resistant; 10.1-30 % of plants infected-Moderately resistant; 30.1-100 % of plants infected-susceptible (Singh et al. 2003; Daspute et al. 2014). The Chi-square ($\chi 2$) test was used to test the goodness of fit of the segregating $F_{2:3}$ families.

C. DNA extraction

The total genomic DNA was isolated from young leaves of individual F_2 plants according to Cetyltrimethyl Ammonium Bromide (CTAB) method described by Murray and Thompson (1986) and DNA was further purified from carbohydrates, proteins and RNA before actual use (Sambrook et al. 1989). The DNA concentration was quantified by using spectrophotometrically (Nanodrop) and accordingly diluted using sterile water for use in PCR assays.

D. Parental screening and identification of polymorphic DNA markers

A set of 300 random decamer DNA markers were screened for parental polymorphism. Out of 300 random markers, 32 primers where polymorphic; among these 13 primers were susceptible parent specific. Further, these 13 polymorphic primers were used for bulked segregant analysis (BSA) (Michelmore et al. 1991) to identify putative linked ones. The two contrasting DNA bulks were made by pooling equal amount of DNA from randomly chosen 8 F_2 homozygous resistant and 8 F_2 homozygous susceptible individuals, respectively. These parental DNA along with the pooled bulk DNA samples were used for RAPD-PCR analysis. Individual components of the resistant bulk and resistant parent were also checked against the susceptible bulk and the susceptible parent for the presence or absence of the characteristic amplicon. Primers showing expected segregation in subsets were tested across all 325 F_2 plants. The marker data scored as presence (1) and absence (0) of characteristic amplicon. This marker data compared with the field phenotypic data for PSMD resistance.

The RAPD-PCR assays were performed similarly according to the explained by Daspute and Fakrudin (2015).

E.Development of SCAR marker

The RAPD amplicon, $IABTPPN18_{827}$ marker, obtained from PSMD susceptible genotype Gullyal white was eluted from 1 % agarose gel and purified using MinElute Gel Extraction Kit (Qiagen, Germany). The purified fragment was cloned into pTZ56 TA cloning vector (Fermentas Life Sciences, EK). The ligation reaction mixture was transformed into fresh prepared competent cells (Sambrook and Russell, 2001) of *Escherichia coli* DH5α strain. The 100 µl of the transformation mixture was spread on the Luria-Bertani

(LB) plates containing 100 µg/ml ampicillin, 20 µl IPTG (100 mM) and 50 µl X-gal and incubated at 37°C overnight. The sequencing was conducted by both ends using universal M 13 forward and reveres primers at the Xcelliries Pvt. Ltd, Ahemadabad, India.

F. Designing of the SCAR primer pairs

Based on the sequence of specific amplification of the loci identified by RAPD markers associated with PSMD responsive gene , two pair of forward and reveres primers were designed with Primer3 tool (http://frodo.wi.mit.edu) with care was taken to avoid possible secondary structure or primer dimer generation, false priming and the melting temperature between pair of primer should not exceed 5°C, to achieve appropriate internal stability while generating SCAR primers. The two SCAR primers sets were synthesized at ILS Pvt. Ltd, Delhi, India.

G. SCAR marker amplification and analysis

PCR amplification was performed in a total volume of 10 μl containing 10 mM Tris–HCl (pH 8.8), 50 mM KCl, 0.1 mM of each dNTP, 0.30 U Taq DNA Polymerase (Bangalore Genei Pvt Ltd), 10 ng of each SCAR primer and 50 ng of template DNA. The PCR programme was as follows: initial denaturation for 4 min at 95°C, followed by 30 cycles each with 20 sec at 94°C, 20 sec at 66.5°C and 30 sec at 72°C, with a final extension of 10 min at 72°C. The PCR products were separated on 1.0 % agarose gels. The molecular weight of the SCAR amplicon estimated with a 100 bp DNA ladder (New England BioLabs).

H. Linkage analysis and SV1-SCAR marker validation

The segregation pattern of the $IABTPPN18_{827}$, $FlnthN18_{827}$ and PSMD reaction among the F_2 population was analysed using MAPMAKER version 3.0 (Lander et al. 1987). A logarithm of odds (LOD) score of 3.0 was used as a linkage threshold. The Kosambi mapping function was used to convert recombination frequency into genetic map distance in centimorgans (cM) Kosambi (1944).Total 96 mini core collection of pigeonpea were field screen during 2011 and 2012 at ARS, Gulbarga to identify their reaction to PPSMV resistance and susceptible, were used for marker validation (Table 4). Kruscal Wallis one way ANOVA was used to study the association between SCAR marker $FlnthN18_{827}$ and PSMD in pigeonpea mini-core collection.

Results

Phenotyping of parents, F_1 and $F_{2:3}$ for PPSMV reaction

Resistance and susceptibility to PSMD of both parents were conformed during 2005, 2006 and 2007. The rows of resistant parent BSMR 736 showed 99% resistance while, the susceptible parent rows of Gullyal White exhibited 98% infection with severe PSMD symptoms. The 23 F_1 seeds of BSMR 736 x Gullyal white were screened for disease reaction. The F1 plants of this cross were susceptible, indicating susceptibility reaction to be dominant over resistant reaction of plants (Table 1).The resulted 325 $F_{2:3}$ families planted in the field were essentially derivative of respective 325 F_2 individual plants. The phenotypic observations for PSMD incidence were recorded according to the disease scale given by Singh et al. (2003). Reaction of $F_{2:3}$ families of BSMR 736 x Gullyal white cross are presented in Fig. 1. A total of 325 $F_{2:3}$ families were field evaluated; 55 showed resistant phenotype, 237 were moderately resistant and 33 families were susceptible for PPSMV Bidar isolate. The disease symptoms including bushy and pale green appearance of leaves, reduction in chlorophyll contain, reduced plant size, increased number of secondary branches, mosaic mottling of leaves and complete or partial cessation of reproductive structures, were categorised as susceptible. Some $F_{2:3}$ families were found to be segregating, among which 70-75 per cent of the progenies were susceptible and rest were resistant. Similarly, a few $F_{2:3}$ families having 100 per cent resistant progenies were also recorded.

Genetic inheritance of PPSMV resistance

The $F_{2:3}$ families showing moderate resistant or susceptible phenotype to PPSMV were combined together into susceptible one category for the convenience. The $F_{2:3}$ families with resistance phenotype was categorised as resistant. The statistical analysis resulted in an observed segregation ratio of 270:55 (susceptible: resistant) ($P < 0.05$), indicating more families with susceptible reaction. The segregation pattern in $F_{2:3}$ families were comparable with 13:3 (susceptible : resistant). Based on the observed segregation ratio it was suggestive that the PPSMV resistance of Bidar isolate is under two gene (*SV1* and *SV2)* control with non-allelic interaction of the type inhibitory gene interaction. The goodness of fit for expected and observed values as tested by $\chi 2$ test with (2.8) and P (3.8) values are presented in Table 2.

RAPD analysis in F_2 population

BSA-RAPD was carried out with 13 parental polymorphic primers, resulting in 2 primers (IABTPPN18 and IABTPPAK19) generating polymorphic amplicon that were present only in susceptible bulk, susceptible

parent and absent in resistance bulk and resistance parent (Fig. 2). Further, IABTPPN18$_{827}$ marker was tested against the subset of resistance and susceptible bulks. The IABTPPN18$_{827}$ marker amplified the polymorphic amplicon only in the susceptible parent and susceptible F_2 segregants and it was clearly absent in resistance parent and resistance F_2 segregants.

Linkage analysis

The RAPD marker IABTPPN18$_{827}$ was used to amplify DNA from all 325 F_2 plants to confirm linkage with PSMD resistance and determine the rate of recombination. The linkage analysis revealed that these markers IABTPPN18$_{827}$ and IABTPPAK19$_{1295}$ where 12.5 and 10 cM away from PSMD responsive gene (*SV1*) respectively.

Identification of SCAR markers lined to the PSMD (SV1) gene

The SCAR primer FlnthN18$_{827}$ produced specific amplicon of 827 bp that is present in susceptible (*SV1SV1sv2sv2*) and absent in resistant (*sv1sv1SV2SV2*) parent, at same molecular size as that of the original RAPD marker IABTPPN18$_{827}$. However, SCAR primer pairs of IABTPPAK19$_{1295}$ RAPD marker failed to give polymorphism with different PCR conditions. The SCAR marker FlnthN18$_{827}$ was screened among all the 325 individuals of the F_2 population. The SCAR marker (FlnthN18$_{827}$) amplified expected susceptible parent specific amplicon in 257 individuals and no-amplification in 68 F_2 individual plants. Segregation of both PSMD resistance and FlanthN18$_{827}$ marker in the F_2 showed that FlanthN18$_{827}$ was present in 254 susceptible plants, absent in 16 susceptible plants, present in 3 resistant plants and absent in 52 resistant plants (Table 3). The ratio for dominant SCAR (FlnthN18$_{827}$) marker was revealed the expected mendelian 3:1 ratio. SCAR marker (FlnthN18$_{827}$) gave the similar pattern as progenitor RAPD marker IABTPPN18$_{827}$ and that was genetically associated with the *SV1* gene.

Validation of SV1- SCAR marker

The *SV1*- SCAR (FlnthN18$_{827}$) was validated across the 96 pigeonpea minicore collection collected from ARS, Gulbarga (Fig. 3, Table 4). SCAR marker FlnthN18$_{827}$ validated for PSMD showed 67.1 per cent association with marker and significant kruskal co-efficient (HC = 33.01, P = 0.0001) (Table 5). The results indicated that SCAR marker FlnthN18$_{827}$ had strong association with sterility mosaic disease as indicated by significant kruskal test co-efficient (HC).

Discussion

Pigeonpea breeding has not achieved the success in sustainable pigeonpea production, because of its susceptible nature to many abiotic and biotic stresses. PSMD one the important biotic threats in pigeonpea breeding program, because of accurate phenotyping, and dynamic nature of PPSMV pathogen. Identification of genetic inheritance of disease is important to know the involvement of gene/s and their interaction governing by the resistance character. In the present study, we identified involvement of two genes (*SV1* and *SV2*) with non-allelic interaction of the type inhibitory gene interaction, governing Bidar PPSMV isolate resistance in BSMR 736 x Gullyal white cross. Similarly, Daspute et al (2014) revealed the similar experimental results in ICP 8863 x BSMR 736 cross for Bidar PPSMV isolate. Amala Balu and Rathnasamy (2003) studied PSMD inheritance in two susceptible parents (Prabath and Co 5), two resistant parents (ICPL 83024 and ICPL 83027). The F_2 generation of the four combinations showed that the segregating ratio of 13:3 for susceptibility and resistance. There are conflicting reports about the genetics of resistance to PSMD claiming both susceptibility and resistance to be dominant. However, in most cases susceptibility was shown to be dominant and resistance to be under the control of recessive genes (Singh et al., 2003). The resistance to PSMD has been reported to be controlled by single recessive gene (Ganpathy et al., 2009; Murugesan et al., 1997; Srinivas et al., 1997) and oligo-genic (Gnanesh et al., 2011; Nagaraj et al., 2004; Sharma et al., 1984). Also, four QTLs for Patancheru PPSMV isolate and two QTLs for Bangalore PPSMV isolate where identified (Gnanesh et al., 2011). Based on our gene interaction hypothesis, we suggest that the dominant allele of *SV1* gene has inhibitory action on the resistance phenotype govern by other *SV2* gene. The proposed genotypes for resistant parent (*sv1sv1SV2 SV2*) and susceptible parent (*SV1 SV1sv2 sv2*), and F_2 generations are [(*SV1-SV2-*): 9; (*SV1-SV2sv2): 3;* (*sv1sv1SV2-): 3;* (*sv1sv1sv2sv2*): 1]. The presence of dominant allele of *SV1* gene in one locus suppresses the action of dominant allele of *SV2* (resistance) gene present on another locus resulting in susceptible phenotype [(*SV1-SV2-*): 9; (*SV1-SV2sv2): 3;* (*sv1sv1sv2sv2*): 1]. Only those plants with recessive allele of *SV1* gene and dominant allele of *SV2* gene might have shown resistant phenotype [(*sv1sv1SV2-): 3]*. Genetics of PSMD has been rely on the resistance source, PSMV isolates and scoring methods; hence the resistance to PSMD in pigeonpea appears to be complex (Saxena, 2008). The results of present experiment revealed that resistance to be governed by two independent non-allelic genes. There may be involvement of additional genes for PSMD resistance in pigeonpea, other than the two proposed in the present study. Recent study of Daspute et al., (2014) revealed involvement of two genes for

PSMD resistance studied in BSMR 736 x ICP 8863 for Bidar isolate and Gnanesh et al. (2011) identified four and two QTLs for Patancheru and Bangalore SMD isolate respectively. Understanding the mode of genetic inheritance of PSMD is expected to aid the pigeonpea breeding and identification DNA markers linked to the PSMD resistance/ susceptibility. RAPD marker have been employed to tag various agronomic traits in pigeonpea such as identification of male sterile and male fertile CMS lines (Souframanien et al. 2003), plant type gene (Dhanasekar et al. 2010), Fusarium wilt resistance gene (Kotreshet et al., 2006) and PSMD resistance gene (Daspute and Fakrudin. 2015). RAPD markers have limitations with large-scale MAS owing to their repeatability and reliability; hence, it is necessary to convert RAPD markers into more robust SCAR markers. The SCAR markers are generally repeatable owing to higher annealing temperatures and longer primer sequence specificity. Although 32 parental polymorphic RAPD primers were subjected for BSA, a primer ($IABTPPN18_{827}$) was successfully converted into a dominant SCAR. Further analysis of individual F_2 plants revealed that SCAR marker $FlnthN18_{827}$, associated with the *SV1* gene and gives same amplification pattern which was revealed by RAPD marker $IABTPPN18_{827}$. Recently Jones et al. (2009) have re-emphasized role of SCAR markers in tracking economically important genes. Failure of other SCAR marker derived from RAPD marker $IABTPPAK19_{1295}$, indicated that original RAPD polymorphisms were caused by mismatches in nucleotides in the priming sites. Similar failures of polymorphic SCAR markers were reported by Paran and Michelmore 1993; Horejsi et al. 1999; Gupta et al. 2006. The prime requirement for MAS in plant breeding program is the identification of linked molecular marker and its validation in different genetic background. Marker validation is the process of investigating the behaviour of trait linked markers and its associated polymorphism in different genetic backgrounds (Gupta et al. 1999; Dhole and Reddy 2013). In addition, 96 minicore collection of pigeonpea with known PSMD reaction where used for validation of the SCAR marker $FlnthN18_{827}$. In different genotypes with known PSMD reaction showed consistent association of the marker in all the PSMD susceptible and resistant genotypes. Some of the resistance and susceptible genotypes of minicore collection were not showed specific amplicon pattern; this may be the reason of recombination between marker flanking site and the (*SV1*) gene responsible for PSMD (Table 4) and or other than *SV1* was exist in minicore population and that may be responsible for PSMD resistance. The results confirm that, the association of this marker with PSMD-*SV1* gene in different genetic backgrounds. The *SV1*-SCAR marker ($FlnthN18_{827}$) associated with a PSMD-*SV1* gene will be useful to discriminate PSMD susceptible genotypes from resistance. Also, it will be useful in mapping resistance gene

and for development of high-yielding PSMD resistant genotypes through MAS without need of artificial screening.

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Table 1. Disease reaction of F_1 against PSMD indicating recessive nature of genes governing resistance in F_1 hybrids.

Bidar isolate	Phenotypic scored
F_1 hybrid	BSMR 736 x Gullyal white
Total plants	23
Susceptible plants	23
Resistant plants	0
Disease incidence (%)	100
Disease reaction	Susceptible

Table 2. Chi-square analyses for segregation of SCAR marker ($FlnthN18_{827}$) associated with the *SV1* gene controlling PSMD resistance in an F_2 population derived from the BSMR 736 x Gullyal white.

Primer Name	No. of F_2 Plants			χ^2 value		
	Present of amplicon	Absence of amplicon	Total	Cal	Tab	Ratio
$FlnthN18_{827}$						
Observed	257	68	325	2.8	3.8	3:1
Expected	244	81	325			

Table 3. Statistical analyses for SCAR marker ($FlnthN18_{827}$) associated with the *SV1* gene of PSMD in an F_2 population derived from the BSMR 736 x Gullyal white.

Marker name	Observed	Expected	χ2 values	
$FlnthN18_{827}$	frequency	frequency	Computed	Table
270^s		$(12^a:1^b)$		
S/+	254	249.2	1.0*	
S/-	16	20.8		
				3.8
55^r		$(0^c:3^d)$		
R/+	3	0	0.2*	
R/-	52	55		

S: PSMD susceptible; R: PSMD resistant; '+': Presence of marker amplicon; '–': Absence of marker amplicon; $^{s:}$ Number of susceptible plant; ^{r}: Number of resistance plant; 12^a: (1) *SV1SV1SV2SV2,* (2) *SV1SV1SV2sv2,* (1) *SV1SV1sv2sv2,* (2) *SV1sv1SV2SV2,* (4) *SV1sv1SV2sv2,* (2) *SV1sv1sv2sv2*- genotypes with presence of *SV1* associated marker; 1^b: (1) *sv1sv1sv2sv2*-genotypes with absence of SV1 associated marker; 0^c: (0)-genotypes with presence of SV1 associated marker; 3^d: (1) *sv1sv1SV2SV2,* (2) *sv1sv1SV2sv2*-genotypes with absence of *SV1* associated marker; *: Non significance at 5% level of significance

Table 4. Amplification status of SCAR marker $FlnthN18_{827}$ in pigeonpea mini -core collection along with accession number and their reaction to PPSMD during 2011-12.

Sl. No.	Accession No.	DR	Amplification status of $FlnthN18_{827}$	Sl. No.	Accession No.	DR	Amplification status of $FlnthN18_{827}$
1	AK-022	S	+	51	GC-11-39	S	+
2	AK-101	S	+	52	GT-1	S	-
3	AKP-1	S	+	53	GT-101	S	-
4	AKT-8811	S	+	54	GULYAL RED	S	+
5	AKT-9913	S	+	55	ICP-8863(MARUTI)	S	+
6	AKT-9915	S	-	56	ICPL-129808	R	-
7	AL-1794	S	+	57	IUPL-332	S	-
8	AL-1855	S	+	58	JAMADAR LOC	S	-
9	AL-201	S	+	59	JKM-189	S	-
10	ASHA (ICPL-87119)	R	-	60	JKM-7	S	-
11	BAHAR	R	+	61	JPB-109B	S	+
12	BANAS	S	+	62	K-2	S	-
13	BDN-2	S	+	63	KARITOGARI	S	-
14	BDN-2004-3	S	-	64	LRG-38	S	+
15	BDN-2008-1	S	-	65	MA-6	R	-
16	BDN-2008-12	S	-	66	MAL-13	S	+
17	BDN-2008-7	S	+	67	NDA-1	S	+
18	BDN-2008-8	S	+	68	PKV-TARA	S	+
19	BDN-2008-9	S	-	69	PG-12	S	+
20	BDN-708	S	+	70	PT-002-2	S	+
21	BENNUR LOC	S	+	71	PT-002251	S	+
22	BIRSA ARHAR-I	S	+	72	PT-04-31	S	-
23	BPG-51-2	S	+	73	PUSA-2001	S	+
24	BPG-51-3	S	+	74	PUSA-9	S	+
25	BRG-11-01	S	+	75	PUSA-991	S	-
26	BRG-109	S	+	76	RVK-272	S	+
27	BSMR-533	S	+	77	RVK-273	S	+
28	BSMR-736	R	-	78	RVK-274	S	+
29	BSMR-853	S	-	79	RVK-275	S	+
30	BWR-153	S	+	80	RVK-277	S	+
31	C-11	S	+	81	RVK-278	S	-
32	CHAPLE	S	+	82	RVK-279	S	+
33	CO-5	S	-	83	RVK-280	S	+
34	CORG-9701	S	+	84	RVK-281	S	+
35	GRG-2009-1	S	+	85	RVK-282	S	+
36	GRG-2009-3	S	+	86	RVK-283	S	-
37	GRG-206	S	+	87	RVK-284	S	+
38	GRG-276-1	S	+	88	RVK-285	S	+
39	GRG-281-1	S	-	89	RVK-286	S	+
40	GRG-333	S	+	90	RVK-287	S	+
41	GRG-815	S	+	91	RVKP-260	S	-
42	GRG-2009	R	+	92	RVKP-261	S	+
43	GRG-2010	S	+	93	RAJA	R	+
44	GRG-822	S	-	94	TS-3R	S	+

45	GRG-2012	S	+	95	TAT-9903	S	+
46	GRG-818	S	-	96	TJT-501	S	+
47	GRG-109	S	+				
48	GRG-107	S	+				
49	GRG-825	S	-				
50	GPHR-08-11	S	+				

DR= PSMD reaction; S= Susceptible; R= Resistant; '+' = Presence of amplicon; '-'= Absence of amplicon

Table 5. Association of SCAR marker with PSMD resistance based on kruskal-wallis one way ANOVA test.

			Kruskal-wallis ANOVA	
SCAR marker	Disease	Per cent Association	HC value	P
$FlnthN18_{827}$	SMD	71.8	15.3**	0.0001

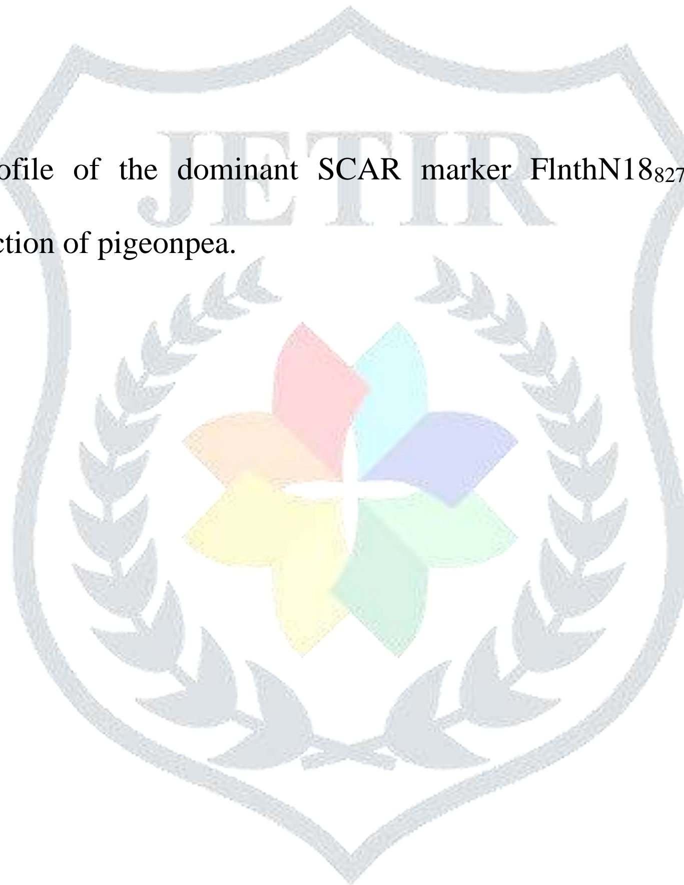
HC = Test co-efficient, **= Significant at P = 0.01

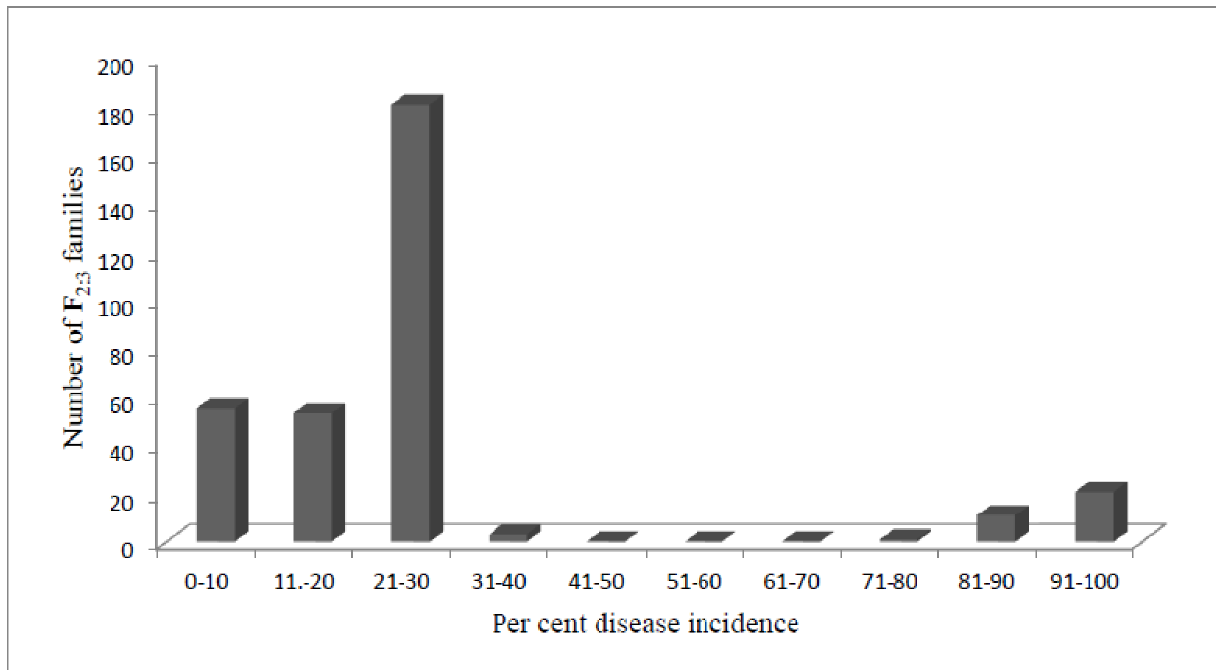
Figure Legends

Fig. 1.- Frequency distribution of per cent disease severity for Bidar PPSMV isolate in 325 $F_{2:3}$ families derived from a cross BSMR 736 x Gullyal white.

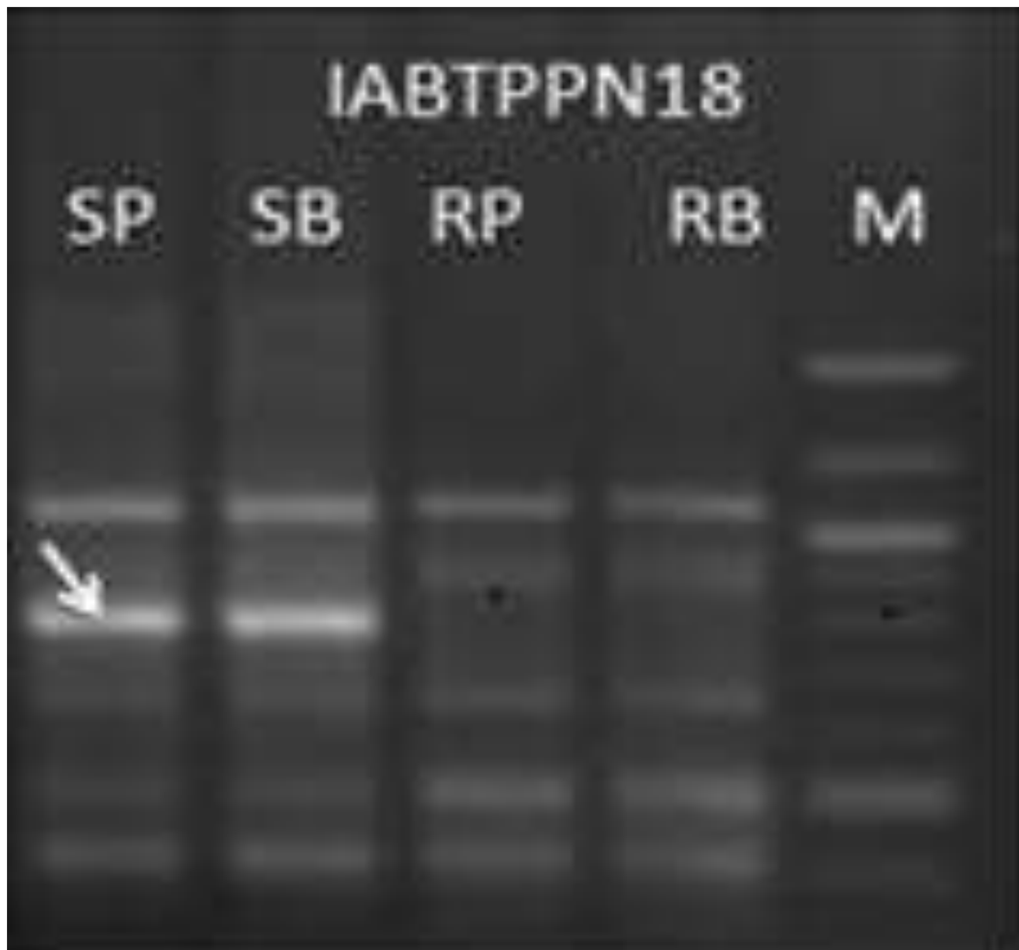
Fig. 2.- Amplification pattern of RAPD marker $IABTPPN18_{827}$ in parents and R and susceptible bulks. M, 100 bp ladder DNA; RP, R parent - BSMR 736; RB, R bulk; SP, susceptible parent - Gullyal white; SB, susceptible bulk.

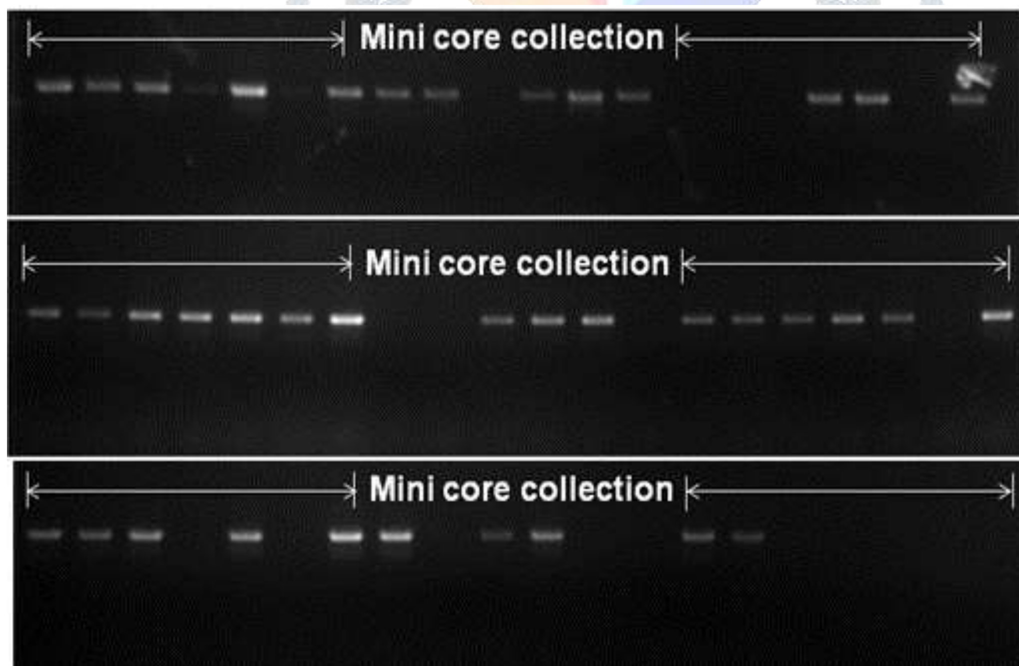
Fig. 3.- Amplification profile of the dominant SCAR marker $FlnthN18_{827}$ in PPSMV resistant and susceptible mini-core collection of pigeonpea.





JETIR


IABTPPN18
SP
SB
RP
RB
M


Mini core collection
Mini core collection
Mini core collection