

# Molecular Markers Linked with Sterility Mosaic Resistance in Pigeonpea (*Cajanus cajan* L. Millsp.)

K.G. Kandarkar<sup>1\*</sup>, N.S. Kute<sup>2</sup>, S.A. Tajne<sup>1</sup>, P.L. Kulwal<sup>3</sup> and K.S. Raghuwanshi<sup>4</sup>

<sup>1</sup>Department of Agricultural Botany, PGI, Mahatma Phule Krishi Vidyapeeth, Rahuri, Ahmednagar 413 722, M.S., India

<sup>2</sup>Pulses Improvement Project, Mahatma Phule Krishi Vidyapeeth, Rahuri, Ahmednagar 413 722, M.S., India

<sup>3</sup>State Level Biotechnology Centre, Mahatma Phule Krishi Vidyapeeth, Rahuri, Ahmednagar 413 722, M.S., India

<sup>4</sup>Department of Plant Pathology and Agricultural Microbiology, Mahatma Phule Krishi Vidyapeeth, Rahuri, Ahmednagar 413 722, M.S., India

(Received 10 February, 2022; Accepted 13 August, 2022)

## ABSTRACT

**Background:** Pigeonpea is an important protein-rich legume and one of the potential crops for rainfed agriculture in many parts of the semi-arid tropics. Worldwide, India ranks first both in area and production. Despite that, the productivity levels in India are low because of various biotic and abiotic stresses, out of which Sterility mosaic disease (SMD), which is caused by Pigeonpea Sterility Mosaic Virus (PPSMV), is a severe constraint during crop production. In the context of the above facts, resistant breeding is one of the most economical and eco-friendly approaches. The present investigation aimed to identify microsatellite markers linked to SMD resistance and potential resistant genetic resources. In this field-laboratory investigation carried out during 2017-2018, thirty genotypes and susceptible check ICP 8863 were evaluated in a randomized block design (RBD) with two replications in SMD sick plots. Observations were recorded based on visual symptoms for SMD incidence, and percent disease incidence (PDI) was calculated. In addition, a molecular study for the same genotypes was carried out with a total of 20 SSR markers. Out of 20 SSR primers, only 16 were amplified, from which only a single SSR marker (AHSSR20) showed polymorphism while the remaining 15 were monomorphic. The marker AHSSR20 have the potential to discriminate resistant and susceptible genotypes. AHSSR 20 primer assign 160 bp bands in resistant genotypes viz., BDN-711, PT-04-281, and PT-704-1-2 while ever, 172 bp bands assign in susceptible genotypes viz. ICP-8863, PT-04-378, PT-012-20, and PT-016-3. Accordingly, we conclude that AHSSR20 is a potent marker that can be helpful to discriminate between susceptible and resistant genotypes and can be used for direct selection of resistant genotypes during early segregating generation and marker-assisted breeding.

**Keywords:** Pigeonpea, Sterility Mosaic, Molecular Marker, SSR, SMD, PDI.

## Introduction

Pigeonpea (*Cajanus cajan* L. Millsp.) is one of the

most important and potential legume crops for rainfed agriculture, particularly in the semi-arid tropics. It belongs to the sub-tribe *Cajaninae*, having

<sup>1</sup>M.Sc., <sup>2,3,4</sup>Ph. D.

a diploid genome with eleven pairs of chromosomes ( $2n = 2x = 22$ ) and genome size around 833.07 Mbp (Greilhuber and Obermayer, 1998, Varshney *et al.*, 2011). It is cultivated either as the sole crop or mixed with maize, sorghum, pearl millet, or with short duration legumes like groundnut in more than 25 tropical and sub-tropical countries. Pigeon pea plays an important role in food security, as a balanced diet, and in alleviating poverty because of its diverse usages as food, fodder, and fuel (Rao *et al.*, 2002). Moreover, it is a versatile crop that is a rich source of protein (20–23%) and thus an important source of income for smallholder farmers. Also, it helps to improve soil fertility by naturally fixing atmospheric nitrogen. Globally, India ranks first both in the area under cultivation (5.06 Mha) and in production (3.29 Mt) (<http://faostat3.fao.org/home/>, as of August 2016). Pigeonpea is the second most important pulse crop next to chickpea in India. In India, during 2019–20 Pigeonpea cultivated over the area of 4.6 Mha and production of around 3.75 Mt with overall productivity of 751 kg/ha (Directorate of Economics and Statistics, 2020 a 3rd advance estimate). At a regional level, Maharashtra ranks second both in respect of area 1.21 Mha and production 0.68 Mt, which contribute to 25.33% and 19.09% of total Pigeonpea area and production in India, followed after Karnataka (1.52 Mha ha and 0.98 Mt) respectively (Directorate of Economics and Statistics, fourth advance estimate, 2018–19).

Despite a more extensive area under Pigeonpea in India, the production levels are low because of various biotic and abiotic stresses. Pigeonpea sterility mosaic virus (PPSMV) caused Sterility mosaic disease (SMD) (Kumar *et al.*, 2000; Jones *et al.*, 2004) is considered to be one of the significant biotic constraints in Pigeonpea cultivation. In India and Nepal, SMD accounts for economic losses of around >US\$ 300 million annually. The SMD virus belongs to the genus Emaravirus (Patil and Kumar, 2015) and is transmitted by an eriophyid mite (*Aceria cajani*, Channabasavanna) (Kulkarni *et al.*, 2002). The characteristic symptoms like the bushy and pale green appearance of plants followed by a reduction in size, increase in the number of secondary branches, and mosaic mottling of leaves during disease development and finally results in a partial or complete cessation of reproductive structures. In some cases, only part of the plant may show symptoms, while other parts may remain unaffected (Kumar *et al.*, 2003).

Among the critical diseases, sterility mosaic disease (SMD) is considered one of the most destructive diseases of Pigeonpea in India and can cause yield loss of up to 95 percent (Reddy and Nene, 1981; Kannaiyan *et al.*, 1984). The development of resistant varieties seems to be quite tricky and complicated because of the genetic plasticity of the pathogen. So, because of such a dynamic nature of the SMD pathogen, there is a need to use strain-specific sources of resistance in crop improvement. Therefore, to develop resistant lines or varieties, there is a need to focus on identifying strain-specific sources of resistance. Also, disease inheritance patterns should be studied for a better understanding.

To reduce crop losses, development for resistant varieties is considered one of the most effective and economical methods that have received top priority as Pigeonpea is a long-duration crop and has an out crossing nature, which creates a problem to screen varieties and breeding populations for SMD resistance. Identification of molecular markers linked to sterility mosaic disease allows screening of cultivars and segregating generations at the seedling stage, which reduces the time and efforts for maintaining virulent isolates of the pathogen and; subsequently, the potential marker can be used in marker-assisted selection. However, due to the scarcity of markers linked to SMD resistance, there is a need to identify tightly linked markers in different Pigeonpea genotypes before applying these markers for marker-assisted selection (MAS) of SMD resistance breeding. In this study, we used 20 molecular markers that screened with thirty-one genotypes to identify a linked marker for SMD resistance and the SMD resistant genotypes in Pigeonpea breeding programs.

## Materials and Methods

### Plant materials

For the present investigation, a set of 31 Pigeonpea genotypes, including one susceptible check obtained from the Pulses Improvement Project, MPKV, Rahuri, was used for the study (Table 1). The experiment was laid in a randomized block design with two replications in SMD sick plots at Pulses Improvement Project, MPKV, Rahuri during *Kharif*, 2017. The gross plot size was taken up as 5 m X 3 m. In each replication, the genotypes were grown in 3 m long rows with 60 × 20 cm spacing for a row to row and plant to plant, respectively. Within a row,

**Table 1.** Sterility Mosaic Percent Disease Incidence (PDI) of different Pigeonpea Genotypes

Sr. No.	Genotype	PDI (%)	Arcsine Transformed	Value Remark
1	ICP-8863 (Check)	33.67	35.47	S
2	PT-0012	28.17	39.91	MR
3	BDN-716	27.27	31.4	MR
4	PT-04-378	31.81	34.33	S
5	PT-705-4-1-2	16.66	24.09	MR
6	ICP-2376	16	23.57	MR
7	Vipula	22.22	28.12	MR
8	ICPL-87	11.54	12.93	MR
9	BDN-711	9.67	18.12	R
10	PT-723-1-2-3	19.35	26.1	MR
11	PT-04-281	9.09	17.54	R
12	PT-704-1-2	9.52	17.97	R
13	PT-711-1-2	18.18	25.23	MR
14	PT-04-111	13.8	21.88	MR
15	PT-04-146-1-2	11.4	19.75	MR
16	PT-04-175	14.28	22.20	MR
17	PT-04-360-1	26.92	31.25	MR
18	PT-04-104-1	25	30.02	MR
19	PT-03-129-2	12.5	20.70	MR
20	PT-012-1	13.79	21.80	MR
21	PT-012-9	16	23.57	MR
22	PT-012-10	21.73	27.79	MR
23	PT-012-11	13.79	21.80	MR
24	PT-012-12	27.27	31.48	MR
25	PT-012-16	19.04	25.87	MR
26	PT-012-20	45.83	42.60	S
27	PT-012-23	24	29.33	MR
28	PT-012-21	23.80	29.20	MR
29	PT-012-27	20	26.56	MR
30	PT-012-22	40.90	39.76	S
31	PT-016-3	30.77	33.69	S

seeds were hand dibbled at 20 cm apart. Genotype ICP-8863 (Maruti) was placed after every five genotypes as SMD susceptible check. A standard package of practices was followed during the experiment to raise the crop.

#### Field evaluations

Screening of genotypes was performed by following the "Leaf Stapling Technique" (Nene and Reddy, 1981). The test entries were phenotyped for resistance to Bangalore SMD isolate at Pulse Improvement Project, Rahuri (Sharma *et al.*, 2015). At the 2-3 leaf stage of the test entries, each of the two primary leaves of the test seedlings was stapled with one or two SMD infected leaves. When the stapled leaflets from the infected plants get dried, mites from the infected leaves migrate to healthy leaves

and inoculate the virus. Incidence of SMD was observed at 15 days intervals up to 75 days done by counting the healthy plants (no mosaic symptoms) and diseased plants (with mosaic symptoms), and scoring was done as per the scale followed in the All India Co-Ordinated Research Project on Pigeonpea for sterility mosaic. The plants were characterized as resistant (0–10% of plants infected), moderately resistant (10.1–30% of plants infected) and susceptible (> 30.1% of plants infected). The percent disease incidence was calculated, and the genotypes were grouped according to disease reaction (Table 2).

#### Extraction and purification of genomic DNA

DNA was extracted from individual plants from the experiment conducted in SMD sick plot. Leaves from 3-4 weeks old plants were collected from the field, sterilized with 70 percent alcohol, and frozen in liquid nitrogen. The leaf samples were stored at –80 °C until further use. The genomic DNA was extracted following the standard CTAB method (Krishna and Jawali, 1997) with minor modifications.

#### PCR Amplification

In the present investigation, a set of twenty SSR primer pairs were used to identify whether these markers are linked to SMD resistance. The primers were selected based on previous studies on SMD resistance in Pigeonpea. The sequence of primers was compiled from the literature and synthesized by Custom Oligo Synthesis Division, Merck Specialties Pvt. Ltd., Bangalore. The details of the SSR primers used in the present investigation are given in Table 3.

The Polymerase chain reaction (PCR) reaction was carried in a 0.2 ml sterile thin-wall PCR tube, and the following components were mixed as each tube (20 µl) consisting 2 µl 50 ng/µl DNA template, 2 pmol of primer, 1.2 µl of 25 mM MgCl<sub>2</sub>, 1.6 µl of 10 mM (2.5mM each) dNTPs mix, 0.2 µl of Genei *Taq* DNA polymerase 5U/µl and 11µl sterilized distilled water.

The PCR reaction was performed in a Korbett Research master cycler in a 96 well plate. Temperature cycling was done by the "Touchdown" method (Mellersh and Sampson, 1993). In touch-down PCR, the amplification of the non-specific sequences can be avoided by adapting high annealing temperature during the earliest steps of a touchdown polymerase chain reaction cycle. Therefore, a touchdown PCR

**Table 2.** Classification of Pigeonpea genotypes based on PDI (%)

PDI (%)	Reaction	Genotypes
0-10	Resistant (3)	BDN-711, PT-04-281, PT-7041-2
10.1-30	Moderately Resistant (23)	PT-0012, BDN-716, PT-705-4-1-2, ICP-2376, Vipula, ICPL-87, PT-723-1-2-3, PT-711-1-2, PT-04-111, PT-04-146-1-2, PT-04-146-1-2, PT-04-175, PT-04-360-1, PT-04-104-1, PT-03-129-2, PT-012-1, PT-012-9, PT-012-10, PT-012-11, PT-012-16, PT-012-23, PT-012-21, PT-012-27
30.1-100	Susceptible (4)	PT-04-378, PT-012-20, PT-012-22, PT-016-3

**Table 3.** List of genic SSR primers used in the present investigation for marker-trait association

Sr. No.	SSR marker	Primer	Sequence (5'-3')	Ta (%C)	Reference
1	CcM0416	F R	AAATTTAAAAATACTCATGTCTGAAGAA CCGTGAGTGTTAATGGACTAATATG	54	B. N. Ganesh., <i>et al.</i> (2011)
2	CcM0468	F R	ATAAAAATATCCGCAACCGC CGAAAGCAATGTCAAAGCAA	56	B. N. Ganesh., <i>et al.</i> (2011)
3	CcM0588	F R	AAAAACAATTATTGCGTAAGATTATCA ACGTTAGGAGCAAAGCGTGT	55	B. N. Ganesh., <i>et al.</i> (2011)
4	CcM0970	F R	TTAAAATCACATCTTACGAAACATAAA AGGACATACGTTCCAAAATTGA	55	B. N. Ganesh., <i>et al.</i> (2011)
5	CcM1447	F R	CTTTCCACGGTCCAGTGAAT TCCATTTGACATGTAAACAATGAAC	56	B. N. Ganesh., <i>et al.</i> (2011)
6	CcM1825	F R	TGAAGTTGGCGGAAAAACAT TCGGACGAAAAACATACTTGC	56	B. N. Ganesh., <i>et al.</i> (2011)
7	CcM1895	F R	GAGGAGAGGAGGCAGAAGGT GTGTCCAGGATTGTAGGGGA	56	B. N. Ganesh., <i>et al.</i> (2011)
8	CcM1982	F R	TATCAAACCTGGCGCATCACA ATTCCGCAAACACATCACAA	54	B. N. Ganesh., <i>et al.</i> (2011)
9	CcM2149	F R	TGTACAGGGCTGTAGGTTCC TCATTTTGACCCTTTTAGATTCC	53	B. N. Ganesh., <i>et al.</i> (2011)
10	CcM2337	F R	TTGTGATAATTTTATATGTGGAAACG GGACCCAGCAAGAAATTTGA	55	B. N. Ganesh <i>et al.</i> (2011)
11	CcM2485	F R	TGTAGAACATGAGTTATTGAACGGA ATTGGGTCCCAGTTTGATTG	57	B. N. Ganesh <i>et al.</i> (2011)
12	CcM2781	F R	TCGTAGTCAAACCAAATCCCT AAAGTGATTTCATCCATAAAAAGTTTG	56	B. N. Ganesh <i>et al.</i> (2011)
13	AHSSR20	F R	AATGTTCTATTGTTTTACGAGTG AATTTCTCGTGTGATTGTGAT	55	P. Patil <i>et al.</i> (2016)
14	AHSSR34	F R	TCTTGAGTAAGTGAACATTCAAA GGTGAAACTCAACTCAACT	54	P. Patil <i>et al.</i> (2016)
15	AHSSR50	F R	AGTTTTTGTGTTTTCAACCTG GAGCAAATAATCATTCAAACAC	55	P. Patil <i>et al.</i> (2016)
16	Ccac036	F R	ATCGGCTTTTGTCTTGATGA AAGCTACAAGGGATACACATGC	53	R.K. Saxena, <i>et al.</i> (2009)
17	CcB10	F R	CCTTCTTAAGGTGAAATGCAAGC CATAACAATAAAAGACCTTGAATGC	56	R. K. Saxena, <i>et al.</i> (2009)
18	CCttc006	F R	GTAGAGGAGGTTCCAAATGACATA ATCTGTCTGGTGTGTTTGTGTGCT	52	R. K. Saxena <i>et al.</i> (2009)
19	CCttc008	F R	TCACAGAGGACCACACGAAG TGGACTAGACATTGCGTGAAG	55	R. K. Saxena <i>et al.</i> (2009)
20	CCttc033	F R	AAATTTAAAAATACTCATGTCTGAAGAA CCGTGAGTGTTAATGGACTAATATG	56	R.K. Saxena <i>et al.</i> (2009)

profile with 4 min initial denaturation cycle followed by first five cycles of 94 °C for 30 secs, 60 °C for 30 secs, 72 °C for 30 secs with 1 °C decrease in annealing temperature depends upon Tm of primer for 30sec and 72 °C for 30 sec followed by final extension for 20 min at 72 °C were adopted.

### Agarose gel electrophoresis

The amplified PCR products were checked on 1.2% agarose gel. The amplified product was fractionated using capillary electrophoresis. Allele sizing of electrophoretic data was obtained from the UV trans-illuminator/ Gel documentation unit (KODAK Molecular Imaging Software with a TWAIN Device).

## Results and Discussion

### Identification of Resistant Genotypes

The observations were recorded on SMD incidence in each plant from 15 DAS to 75 DAS. The per cent disease incidence was calculated based on morphological symptoms, and genotypes were grouped according to a standard scale for PDI into the susceptible, moderately resistant, and resistant categories. In this investigation, out of 30 genotypes along with check ICP 8863, the total genotypes were categorized as 3 (10%) resistant (R), 23 (76.66%) moderately resistant (MR), and 4 (13.33%) susceptible (S).

Out of thirty genotypes, only three genotypes viz., BDN-711, PT-04-281, and PT-7041-2 showed resistance to SMD. A similar kind of results was reported in earlier studies where 52 AICRP Pigeonpea genotypes and checks were screened for SMD, and out of that, only eight entries viz. ICPL-87119, ICPL-2376, BDN-2, PT-4-307, CORG-9701, BSMR-736, GRG-811, and BSMR-853 were resistant entries (Bhaskar, 2016). In the past study, 61 Hyderabad accessions were tested against SMD, only two entries viz. ICPL99095 and ICP7035 showed a resistant reaction, and only a single entry ICPL20123 showed moderately immune response while the rest of the entries were showed susceptible responses (Prabhavathi *et al.*, 2018). Therefore, genotypes (BDN-711, PT-04-281, and PT-7041-2) screened out in the present study can be used as a potent source of SMD resistance in future hybridization programs. From the observations, it was clear that many plants were classified into moderately resistant and susceptible groups. While only a few plants were classified into the resistant group. It indicates a need to

find the more potent source for SMD resistance as there is the involvement of a large number of segregating genes, with the majority of them having increasing effects.

### Molecular analysis

Agarose gel electrophoresis showed polymorphism in the DNA banding pattern. Out of 20 SSR primers, only 16 were amplified, and only a single primer (AHSSR20) showed polymorphism while the remaining 15 were monomorphic. The size of the amplified product ranged from 91-389 bp (Table 4). Similar reports were also reported where 32 Pigeonpea accessions were screened with 30 microsatellite markers; out of that 23 showed polymorphism (Saxena *et al.*, 2009).

The marker profile of AHSSR 20 was helpful to

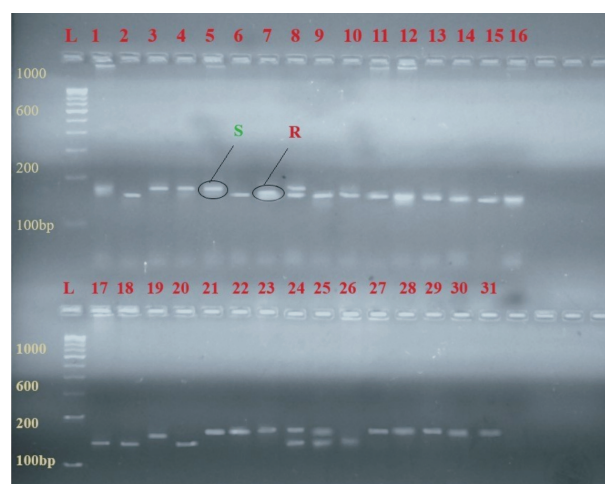
**Table 4.** Details of the SSR primers used for amplification of genomic DNA of Pigeonpea

Sr. No.	Particulars	Observation
1.	Total number of primers used	20
2.	Number of primers amplified DNA	16
3.	Total number of polymorphic markers	1
4.	Percentage of polymorphic markers	6.25%
5.	Size of the amplified product range	91-389 bp

differentiate between SMD susceptible and resistant genotypes. Among the primers, the only primer AHSSR20 was found linked to SMD resistance in Pigeonpea. Only one SSR marker, i.e. AHSSR20 amplified PCR product of 160 bp in resistant genotypes viz., BDN-711, PT-04-281, and 172 bp present in susceptible genotypes viz. ICP-8863, PT-04-378, PT-012-20, PT-016-3 (Fig.1) may be useful for a marker-trait association for SMD resistance in studied genotypes. Rest markers were not able to distinguish the resistant and susceptible genotypes. The Primer AHSSR20 shows a good variation between resistance and susceptible genotypes.

In some genotypes (ICPL-87, PT-012-12, and PT-012-16), both bands were reported which shown moderate resistance based on PDI, which are in agreement with earlier studies where it was reported that dominant genes govern susceptibility, while resistance under recessive gene control (Nagraj *et al.* 2004 and Ganapathy *et al.*, 2009).

In the present investigation, the information obtained from the molecular profile of AHSSR 20 correlates with field data as that of field PDI data and



**Fig. 1.** SSR primer AHSSR20 based DNA polymorphism of Pigeonpea genotype

**L= 100bp ladder**

Lane	Genotypes	Lane	Genotypes
1	PT-0012	16	PT-04-175
2	BDN-716	17	PT-04-360-1
3	PT-04-378	18	PT-04-104-1
4	PT-705-4-1-2	19	PT-03-129-2
5	ICP-8863 (Check)	20	PT-012-1
6	ICP-2376	21	PT-012-9
7	Vipula	22	PT-012-10
8	ICPL-87	23	PT-012-11
9	BDN-711	24	PT-012-12
10	PT-723-1-2-3	25	PT-012-16
11	PT-04-281	26	PT-012-20
12	PT-704-1-2	27	PT-012-23
13	PT-711-1-2	28	PT-012-21
14	PT-04-111	29	PT-012-27
15	PT-04-146-1-2	30	PT-012-22
		31	PT-016-3

marker profile had shown nearly similar pattern to discriminate lines for resistance and valuable in the identification of susceptible and resistant lines. So AHSSR20 needs to be validated through Bulk Segregant Analysis (BSA) or Single Marker Analysis (SMA) by using proper mapping populations like RIL or NIL, and after that, it can be used in the future breeding program for transferring SMD resistant gene/QTL to susceptible lines through marker-assisted breeding.

In the previous study, based on the similarity coefficient for inter-genotype, seven genotypes were clustered into different groups with the help of 61 SSR markers which segregate genotypes clearly as resistant and susceptible categories. This study supports the potential application of microsatellite

markers in the differentiation of genotypes with a contrasting expression for response to Pigeonpea sterility mosaic disease infection. They had reported thirteen out of 46 polymorphic markers like CCttc003, CCttc006, CCttc008, CCat011, CCtc012, CCttc033, CCac036, ICPM1D10, ICPM1E04, ICPM1H01, ICPM2B08, PB1, and PB12 could be helpful in discrimination of SMD resistant and susceptible genotypes into different groups (Naik *et al.* 2012).

Cultivated Pigeonpea is understood to possess shallow genetic diversity exhibited at the DNA level (Yang *et al.*, 2006). Hence, it is vital to generate additional genomic resources, especially SSR and SNP markers, for their utilization in molecular breeding. Identifying DNA markers linked to important traits such as Fusarium wilt, SMD, submergence, and drought tolerance will go a long way in the Pigeonpea improvement. Most of these traits remain to be studied in detail for their inheritance and trait governance. Reasonably often, not breeders/researchers develop mapping populations involving parents contrasting for the trait(s) targeted for mapping without considering their polymorphism level.

## Conclusion

The present study was conducted with objectives, identification of molecular markers linked with SMD resistance and potential resistant genotypes. In the present study, 30 genotypes and a susceptible check were screened with 20 microsatellite markers. Out of the 20, SSRs used, only 16 get amplified, while the single SSR (AHSSR 20) was polymorphic. Therefore, it can be effectively used to characterise Pigeonpea genotypes for SMD resistance and susceptibility. Out of thirty genotypes, only three viz., BDN-711, PT-04-281, and PT-704-1-2 were identified as resistant to SMD based on a single SSR (AHSSR 20) amplified product of 160bp. Conclusively, AHSSR 20 marker will be helpful for direct selection of resistant Pigeonpea genotypes in early segregating generations and marker-assisted backcross breeding for SMD resistance.

## Acknowledgement

The authors are grateful to the Department of Agricultural Botany, Pulses Improvement Project and State Level Biotechnology Centre, MPKV, Rahuri

(MS) for the facilities provided and supply of seed material of Pigeonpea accessions for the present study.

### Author'S Contribution

N.S. Kute and P.L. Kulwal designed the experiments. K.G. Kandarkar conducted an investigation involved in genotyping and field screening. K.S. Raghuwanshi helped in data analysis for screening. K.G. Kandarkar and S. A. Tajnewrote the paper. N.S. Kute and P.L. Kulwal reviewed the manuscript. All authors read and approved the manuscript.

### References

- Bhaskar, A.V. 2016. Screening of pigeonpea genotypes against wilt and sterility mosaic disease in Telangana state, India. *Indian J. Agric. Res.* 50 (2): 172-176.
- Ganapathy, K. N., Byre Gowda, M., Venkatesha, S. C., Rama Chandra, R., Ganesh, B. N. and Girish, G. 2009. Identification of AFLP markers linked to sterility mosaic disease in Pigeonpea *Cajanus cajan* (L.) Millsp. *IJIB*. 7 : 145-149.
- Greilhuber, J. and Obermayer, R. 1998. Genome size variation in *Cajanus cajan* (Fabaceae): a reconsideration. *Plant Syst. Evol.* 212 : 135-141.
- Jones, T., Lava Kumar, I.K., Saxena, B. Kulkarni, N.K. and Muniyappa, V. 2004. Sterility mosaic disease – the “Green Plague” of Pigeonpea University of Agriculture Sciences, Bangalore, India. *Farid Waliyar ICRISAT Plant Disease / Vol. 88 No. 5.*
- Krishna, T.G. and Jawali, N. 1997. DNA isolation from single or half seeds suitable for random amplified polymorphic DNA analysis. *Analytical Biochem.* 250: 125-127.
- Kulkarni N.K., Kumar P.L., Muniyappa V., Jones A.T. and Reddy D.V.R. 2002. Transmission of Pigeonpea sterility mosaic virus by the eriophyid mite, *Aceriacajani* (Acari: Arthropoda). *Plant Dis.* 86 : 1297-1302.
- Kumar, P.L., Jones, A.T., Sreenivasulu, P. and Reddy, D.V.R. 2000. Breakthrough in the identification of the causal agent of Pigeonpea sterility mosaic disease. *J. Mycol. Plant Pathol.* 30: 249.
- Mellersh, C. and Sampson, J. 1993. Simplifying detection of microsatellite length polymorphisms. *Bio Techniques.* 15: 582-584.
- Nagaraj, K.M., Chikkadevaiah and Kulkarni, R.S. 2004. Inheritance of resistance to sterility mosaic virus in Pigeonpea (*Cajanus cajan* (L.)). *Indian J. Genet.* 64(2) : 118-120.
- Naik, S., M. Byre Gowda<sup>1</sup>, S.C. Venkatesha, H.K. Ramappa, C.K. Pramila, G.A. Mary Reena and Ramesh, S. 2012. Molecular diversity among pigeonpea genotypes differing in response to PSMD. *Journal of Food Legumes.* 25(3): 194-199.
- Patil, B.L. and Kumar, P.L. 2015. Pigeonpea sterility mosaic virus: a legume-infecting Emaravirus from South Asia. *Mol. Plant Pathol.* 13: DOI:10.1111/mpp.12238.
- Patil, P. G., Byregowda, M., Bhuvaneshwara, R. Patil, Alok Das, Mary Reena Shashidhar, H.E. 2016. Microsatellite markers linked to sterility mosaic disease resistance in Pigeonpea (*Cajanus cajan* L. Millsp.). *Legume Genomics and Genetics.* 7 (6) : 1-9.
- Prabhavathi, N.M. and Ramappa, H.K. 2018. Field Evaluation of Pigeonpea Genotypes against Pigeonpea Sterility Mosaic Virus (PPSMV). *Int. J. Pure App. Biosci.* 6(5) : 57-61 DOI: <http://dx.doi.org/10.18782/2320-7051.6874>
- Rao, S.C., Coleman, S.W. and Mayeux, H.S. 2002. Forage production and nutritive value of selected Pigeonpea ecotypes in southern Great Plains. *Crop Sci.* 42: 12591263.
- Reddy, M.V. and Nene, Y.L. 1981. Estimation of yield loss in Pigeonpea due to Sterility Mosaicin *Proceedings of International workshop on Pigeonpeas.* 2 : 15-19 December (1980), ICRISAT centre. Patancheru, Andhra Pradesh, India. pp. 305-312.
- Saxena, R.K., Saxena, K., Kumar, R.V., Hoisington, D.A. and Varshney, R.K. 2009. Simple sequence repeat-based diversity in elite pigeonpea genotypes for developing mapping populations to map resistance to *Fusarium* wilt and sterility mosaic disease. *Plant Breed.* 1-7.
- Sharma, M., Telangre, R. and Ghosh, R. 2015. Multi-environment field testing to identify broad, stable resistance to sterility mosaic disease of pigeonpea. *J Gen Plant Pathol.* 81: 249-259. <https://doi.org/10.1007/s10327-015-0585-z>
- Varshney, R.K., Chen, W., Li, Y., Bharti, A. K., Saxena, R.K., Schlueter, J.A., Donoghue, M.T., Azam, S., Fan, G., Whaley, A.M., Farmer, A.D., Sheridan, J., Iwata, A., Tuteja, R., Penmetsa, R.V., Wu, W., Upadhyaya, H.D., Yang, S. P., Shah, T., Saxena, K.B. and Jackson, S.A. 2011. Draft genome sequence of pigeonpea (*Cajanus cajan*), an orphan legume crop of resource-poor farmers. *Nature Biotechnology.* 30(1): 83-89. <https://doi.org/10.1038/nbt.2022>
- Yang, S., Pang, W. and Ash, G. 2006. Low level of genetic diversity in cultivated Pigeonpea compared to its wild relatives is revealed by diversity arrays technology. *Theor Appl Genet.* 113: 585-595. <https://doi.org/10.1007/s00122-006-0317-z>