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# IDENTIFICATION OF STERILITY MOSAIC DISEASE (SMD) RESISTANCE VEGETABLE PIGEONPEA HYBRIDS (*CAJANUS CAJAN* L. MILLSP.)

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**ABSTRACT**

Sterility Mosaic Disease (SMD) is one of the most destructive diseases affecting pigeonpea in the Indian subcontinent, leading to a significant reduction in yield. The development of resistant varieties or hybrids is considered an effective strategy for managing this disease. Therefore, the primary goal of this study was to identify SMD resistant vegetable pigeonpea hybrids and investigate the relationship between SMD resistance and certain leaf characteristics, specifically leaf colour and texture. During *Kharif*-2018, a total of 24 $F_1$ hybrids, 10 parents (comprising 4 lines and 6 testers) and 2 control varieties (ICP 7035, a resistant check, and ICP 8863, a susceptible check) were evaluated for their response to SMD. Among the evaluated Parents, the lines BRG 3 exhibited 100% resistance to SMD, with no visible symptoms, while BRG 5, Hy3C and ICPL 20325 were found to be moderately resistant to SMD. Among the tested hybrids, a single hybrid (ICPL 20325 × Hy3C), categorized as moderately resistant × moderately resistant, exhibited resistance to SMD with a *Percent* Disease Index or Disease Severity Index (PDI) of 9.09. This suggests that the hybrid inherited resistance genes from both of its parent plants. Additionally, hybrids created from crosses such as ICPL 87091 × BRG 3, ICPL 20325 × BRG 3, BRGL 24-2 × BRG 3, and BRG 4 × BRG 3, classified as moderately resistant × resistant, demonstrated SMD resistance with PDIs of 8.33, 4.16, 8.0 and 4.76, respectively. Notably, these hybrids included BRG 3 (a resistant parent), indicating monogenic resistance to SMD. Visual observations of leaf colour and texture revealed that SMD resistant genotypes had dark green leaves with a leathery texture, while SMD-susceptible genotypes had light green leaves without a leathery texture. This suggests that specific leaf traits are linked to SMD resistance in the studied parent plants. If further confirmed, the potential association between dark green leathery leaves and SMD resistance could have substantial implications for enhancing pigeonpea crops by enabling the indirect selection of these leaf traits in the development of SMD resistant cultivars.

***Key words :*** Pigeonpea, Sterility Mosaic Disease (SMD), Resistance, Leaf colour, Leaf texture.

## Introduction

Pigeon pea (*Cajanus cajan*), also known as redgram, arhar, or tur, is a leguminous plant belonging to the Fabaceae family. Having been domesticated in the Indian subcontinent over 3500 years ago, its seeds have become a staple food in Asia, Africa and Latin America. It is extensively consumed in South Asia and serves as a vital protein source for the population of the Indian subcontinent. Globally, pigeon pea cultivation spans 63.57 lakh hectares, resulting in a production of 54.75 lakh tonnes with productivity rate of 861.25 kg/ha, according to FAO STAT (Anonymous, 2021). India holds a prominent position as the global leader in redgram production, contributing 43.40 lakh tonnes from 49.80 lakh hectares in the 2021-22 seasons, with a productivity of 871 kg/hectare as documented (Anonymous-agricoop.nic.in.). In India, pigeon pea ranks second in terms of both area and production, following chickpea. Notably, the states of Maharashtra and Karnataka are at the forefront of production, occupying 12.98 and 12.40 lakh hectares, respectively.

In recent years, pigeonpea has gained significance for its adaptability to challenging agricultural conditions, serving as both a food source and a forage or cover crop. It is particularly known for its high-protein split pulses, commonly consumed as 'dhal.' The tender green pods of pigeonpea have become a favored vegetable, especially in India and in several African nations, where it is cultivated exclusively for vegetable processing industries.

Vegetable pigeonpea varieties, characterized by larger pods and seeds, offer superior nutritional value compared to dhal-type pigeonpeas, featuring higher crude fiber, fat, and protein digestibility. Notably, they excel in mineral and trace element content, including phosphorus, potassium, zinc, copper, and iron (Singh *et al*., 1984).

Despite its nutritional benefits, the production of vegetable pigeonpea faces challenges such as limited availability of suitable cultivars and the prevalence of severe biotic and abiotic stresses, including Fusarium wilt, Sterility Mosaic Disease (SMD) and Phytophthora blight (Reddy *et al*., 1998). SMD, caused by the pigeonpea sterility mosaic virus (PPSMV) transmitted by the Eriophyid mite vector *Aceria cajani* (Kumar *et al*., 2003), is a major concern causing significant yield losses.

Efforts to manage SMD through chemical methods are often economically impractical under resource-constrained conditions. Therefore, developing SMD-resistant varieties is a promising strategy, contingent upon a comprehensive understanding of the genetics underlying SMD resistance.

Despite extensive research on SMD resistance, there is no consensus on whether resistance is conferred by recessive or dominant genes. The precise mechanisms remain elusive, with studies suggesting a monogenic segregation ratio and co-segregation with specific leaf characteristics (Murugesan *et al*., 1997). Other studies propose that the resistance observed in the ICP 7035 variety results from a thick leaf cuticle acting as a barrier against mite penetration (Reddy *et al*., 1995).

Addressing the challenge of limited yield improvements in pigeonpea, this study aims to identify SMD-resistant vegetable pigeonpea hybrids and explore the potential association between SMD resistance and specific leaf traits, including colour and texture. The development of SMD-resistant parental lines, coupled with cytoplasmic genic male sterility systems, holds promise for significantly enhancing pigeonpea productivity (Saxena *et al*., 2006).

## Materials and Methods

The investigation was conducted at the experimental plot of the All India Co-ordinated Research Project (AICRP) on Pigeonpea, located at the Zonal Agricultural Research Station (ZARS), University of Agricultural Sciences (UAS), Gandhi Krishi Vignana Kendra (GKVK), Bengaluru. The material for the study consisted of 24 $F_1$ hybrids were produced by crossing four parental lines (ICPL 87091, ICPL 20325, BRG 4 and BRGL 24-2) and six tester lines (BRG 1, BRG 3, BRG 5, BRGL 9-2, BRGL 13-8 and Hy3c) using the line × tester mating design recommended by Kempthorne (1957) during the *Kharif*-2017 season. Additionally, 10 parental lines, along with the resistant check (ICP 7035) and the susceptible check (ICP 8863), were incorporated in the study materials. The breeding lines were sourced from AICRP on Pigeonpea UAS, GKVK, Bengaluru, and the International Crop Research Institute for the Semi-Arid Tropics (ICRISAT), Hyderabad (Table 1).

During *Kharif*-2018, all the 24 hybrids, 10 parental lines, and the resistant check ICP 7035 and susceptible check ICP 8863, underwent field evaluation. The evaluation was conducted in a randomized complete block design (RCBD) with two replications with a spacing of 90 × 60 cm between rows and individual plants respectively in a Sterility Mosaic Disease (SMD) infested plot (ICPL 8863 as the infector row). SMD resistance was assessed using the standard leaf stapling method as outlined by Nene and Reddy (1977).

**Leaf Stapling Technique :** We strictly followed the methodology outlined by Nene *et al.* (1977). An SMD-infected leaflet with a significant mite population was stapled onto the healthy, uninfected leaves of 10-15 days old seedlings of each hybrids, parental lines, resistant check and susceptible check. Infected leaves were examined under a binocular microscope to confirm the presence of eriophyid mites. To achieve inoculation at the primary leaf stage and rapid symptom expression, the diseased leaflets were securely folded over the primary leaf, ensuring contact between the lower surface of the infected leaf and the primary leaf. Leaflets were then stapled in place using a small paper stapler. In cases of smaller diseased leaves, two leaves were used alternatively, maintaining contact between the lower surface of the infected leaf and both surfaces of the test plant's leaflet. This method facilitated efficient inoculation at the primary leaf stage, enabling swift disease symptom

**Table 1 :** Salient features of pigeonpea parental lines used in this study.

| S. no. | Genotypes | Salient features |
|---|---|---|
| 1 | BRG 1 | Bold seeded, dual purpose, moderately resistant to SMD |
| 2 | BRG 3 | SMD and wilt resistant, Mottled seeds, dual purpose |
| 3 | BRG 4 | Mid early duration, brown seeded |
| 4 | BRG 5 | Resistant to wilt, moderately resistant to SMD |
| 5 | BRGL 9-2 | Bold seeded, wilt resistant |
| 6 | BRGL 13-8 | Bold seeded, wilt resistant |
| 7 | BRGL 24-2 | Early maturing, wilt resistant |
| 8 | ICPL 20325 | Early maturing, bold seeded and moderately resistant to SMD and wilt |
| 9 | ICPL 87091 | Early maturing, bold seeded |
| 10 | Hy3C | Bold and white seeded, moderately resistant to SMD |
| C1 | ICP 7035 | Early maturity, SMD resistant |
| C2 | ICP 8863 | SMD susceptible |

**Source:** Annual Report 2011, AICRP on Pigeonpea, UAS, Bengaluru.

**Table 2 :** Scale adopted for categorizing pigeonpea genotypes against SMD.

| Disease reaction | SMD incidence (%) |
|---|---|
| Resistant | 0-10 % |
| Moderately resistant | 10-30 % |
| Susceptible | > 30 % |

**Source:** AICRP on Pigeon pea, ZARS, UAS, GKVK, Bengaluru.

expression. It proved valuable for confirming resistance observed under field conditions and for studies on disease inheritance and strain identification.

As the stapled leaflets from infected plants dried, mites migrated to healthy leaves, initiating virus transmission. Following virus transmission, seedlings were monitored for SMD incidence at 15-day intervals up to 75 days. Monitoring involved counting healthy plants (lacking mosaic symptoms) and diseased plants (displaying mosaic symptoms), following criteria established by the All India Coordinated Research Project (AICRP) on pigeonpea. Percent disease incidence (PDI) was calculated based on regular symptom monitoring, categorizing hybrids into different disease reactions (Table 2).

Disease incidence (%) was calculated using formula:

$$\text{Disease incidence (\%)} = \frac{\text{Number of plant infected}}{\text{Total number of plants inoculated}} \times 100$$

## Results and Discussion

**Sterility Mosaic Disease (SMD) Resistance :** Among the evaluated Parents, the lines BRG 3 exhibited 100% resistance to SMD with no visible symptoms, while BRG 5, Hy3C and ICPL 20325 were found to be moderately resistant to SMD (Table 3a). Similarly, the resistant check ICP7035 recorded 100% resistance to SMD, with no visible symptoms where as susceptible check ICP 8863 displayed 100% infection with severe mosaic symptoms (Table 3b, Fig. 1a). These SMD resistant lines can be valuable contributors to breeding programs aimed at developing high-yield, SMD-resistant hybrids. These results are consistent with previous research findings, such as those of Saifulla *et al*. (2006), who conducted a multi-year screening of four pigeonpea genotypes (BRG 3, ICP 7035, Hy-3C and ICP 8863) for SMD resistance. They found that genotypes BRG 3 and ICP 7035 exhibited a resistant reaction. Additionally, Rangaswamy *et al*. (2005) reported that ICPL 7035 displayed resistance to SMD.

Among the evaluated pigeonpea hybrids, those originating from the crosses ICPL 87091 × BRG 3, ICPL 20325 × BRG 3, BRGL 24-2 × BRG 3 and BRG 4 × BRG 3, which fall into the category of moderately resistant × resistant, displayed notable resistance to Sterility Mosaic Disease. They exhibited Disease Severity Index (PDI) values of 8.33, 4.16, 8.0 and 4.76, respectively (Table 4). Additionally, a single hybrid, ICPL 20325 × Hy3C, belonging to the class of moderately resistant × moderately resistant, demonstrated robust resistance to SMD, with a PDI of 9.09 (Fig. 1b). This implies that resistance genes were inherited from both parent plants in this hybrid. In contrast, the remaining crosses exhibited moderate resistance to SMD, with PDIs ranging from 10% to 30%. The resistance observed in these crosses is likely attributable to the transfer of resistant genes during the hybridization program.

**Association of Leaf Colour and Texture with Resistance :** Visual examination of resistant and moderately resistant hybrids& parents revealed dark green, leathery leaves, while the susceptible line (ICP

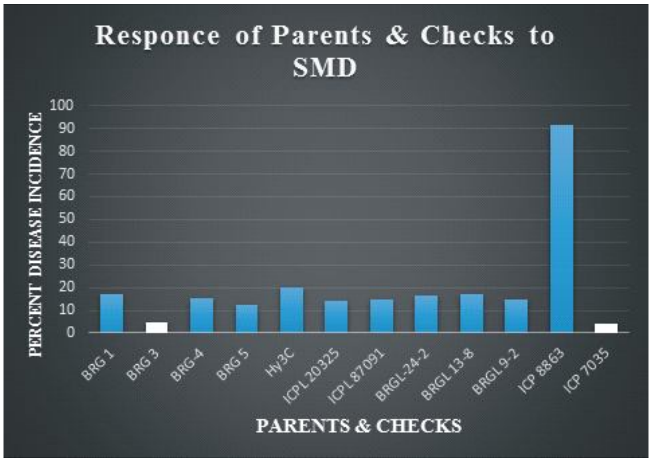


**Fig. 1a :** Reaction of Parents & checks on SMD infection.


**Plate 1(a) :** Expression of resistance in cross BRG-4 × BRG-3.

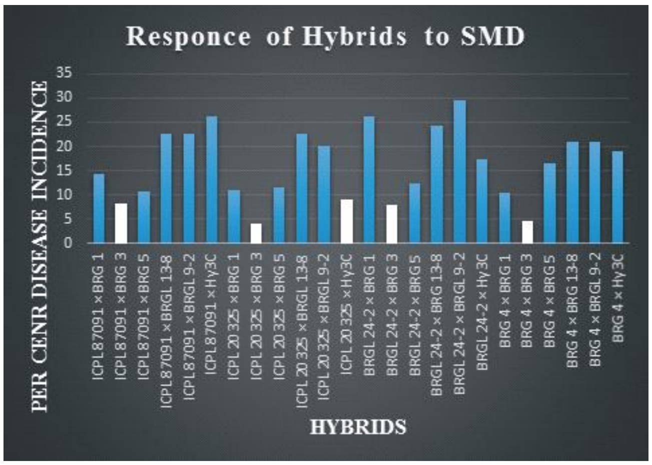


**Fig. 1b :** Reaction of Hybrids on SMD infection.


**Plate 1(b) :** SMD susceptible check ICP 8863.

**Table 3a :** Reaction of Parents to Sterility Mosaic Disease Infection in the study.

| Parents | Total no. of plants | No. of diseased plants | No. of healthy plants | PDI (%) | Disease Response |
|---|---|---|---|---|---|
| BRG 1 | 23 | 4 | 19 | 17.39 | Moderately resistance |
| BRG 3 | 22 | 1 | 21 | 4.54 | Résistance |
| BRG-4 | 26 | 4 | 22 | 15.38 | Moderately resistance |
| BRG 5 | 24 | 3 | 21 | 12.50 | Moderately resistance |
| Hy3C | 20 | 4 | 16 | 20.00 | Moderately resistance |
| ICPL 20325 | 21 | 3 | 18 | 14.28 | Moderately resistance |
| ICPL 87091 | 20 | 3 | 17 | 15.00 | Moderately resistance |
| BRGL-24-2 | 24 | 4 | 20 | 16.66 | Moderately resistance |
| BRGL 13-8 | 23 | 4 | 21 | 17.39 | Moderately resistance |
| BRGL 9-2 | 20 | 3 | 17 | 15.00 | Moderately resistance |

8863) exhibited light green, non-leathery leaves (Plate 1a and Plate 1b). This observation was consistent with ICP 7035, displaying dark green, leathery leaves linked to SMD resistance, while ICP 8863 exhibited light green, non-leathery leaves associated with susceptibility (Plate 1c).

These results align with prior studies indicating a genetic correlation between SMD resistance and leaf morphological traits (Murugesan *et al.*, 1997). Reports by Reddy *et al.* (1995) supported our findings, highlighting thicker leaf cuticles and epidermal cell walls in SMD-resistant genotypes compared to susceptible ones. Our investigation of ICP 7035 and ICP 8863 mirrored Reddy *et al.* (1995) study, revealing ICP 7035's thicker leaf cuticle (3.79 μm) compared to ICP 8863 (2.27μm). The

**Table 3b :** Reaction of Checks to Sterility Mosaic Disease Infection in the study.

| Checks | Total no. of plants | No. of diseased plants | No. of healthy plants | PDI (%) | Disease Response |
|---|---|---|---|---|---|
| **ICP 8863** | 24 | 21 | 02 | 91.66 | **Susceptible** |
| **ICP 7035** | 25 | 1 | 24 | 4.00 | **Résistance** |

**Table 4 :** Reaction of hybrids to sterility mosaic disease infection in the study.

| Hybrids | Total no. of plants | No. of diseased plants | No. of healthy plants | PDI (%) | Disease Response |
|---|---|---|---|---|---|
| ICPL 87091 × BRG 1 | 21 | 3 | 18 | 14.28 | Moderately resistance |
| ICPL 87091 × BRG 3 | 24 | 2 | 22 | 8.33 | Résistance |
| ICPL 87091 × BRG 5 | 28 | 3 | 25 | 10.71 | Moderately resistance |
| ICPL 87091 × BRGL 13-8 | 22 | 5 | 17 | 22.72 | Moderately resistance |
| ICPL 87091 × BRGL 9-2 | 22 | 3 | 19 | 22.72 | Moderately resistance |
| ICPL 87091 × Hy3C | 23 | 6 | 17 | 26.08 | Moderately resistance |
| ICPL 20325 × BRG 1 | 18 | 2 | 16 | 11.11 | Moderately resistance |
| ICPL 20325 × BRG 3 | 24 | 2 | 22 | 4.16 | Résistance |
| ICPL 20325 × BRG 5 | 26 | 1 | 25 | 11.53 | Moderately resistance |
| ICPL 20325 × BRGL 13-8 | 22 | 3 | 19 | 22.72 | Moderately resistance |
| ICPL 20325 × BRGL 9-2 | 20 | 5 | 15 | 20.00 | Moderately resistance |
| ICPL 20325 × Hy3C | 22 | 4 | 18 | 9.09 | Résistance |
| BRGL 24-2 × BRG 1 | 23 | 6 | 17 | 26.08 | Moderately resistance |
| BRGL 24-2 × BRG 3 | 25 | 2 | 23 | 8.00 | Résistance |
| BRGL 24-2 × BRG 5 | 32 | 2 | 30 | 12.50 | Moderately resistance |
| BRGL 24-2 × BRG 13-8 | 25 | 5 | 20 | 24.40 | Moderately resistance |
| BRGL 24-2 × BRGL 9-2 | 27 | 4 | 23 | 29.62 | Moderately resistance |
| BRGL 24-2 × Hy3C | 29 | 8 | 21 | 17.24 | Moderately resistance |
| BRG 4 × BRG 1 | 19 | 5 | 14 | 10.52 | Moderately resistance |
| BRG 4 × BRG 3 | 21 | 2 | 19 | 4.76 | Résistance |
| BRG 4 × BRG 5 | 18 | 1 | 17 | 16.66 | Moderately resistance |
| BRG 4 × BRG 13-8 | 24 | 3 | 21 | 20.83 | Moderately resistance |
| BRG 4 × BRGL 9-2 | 19 | 5 | 14 | 21.05 | Moderately resistance |
| BRG 4 × Hy3C | 24 | 4 | 20 | 19.04 | Moderately resistance |

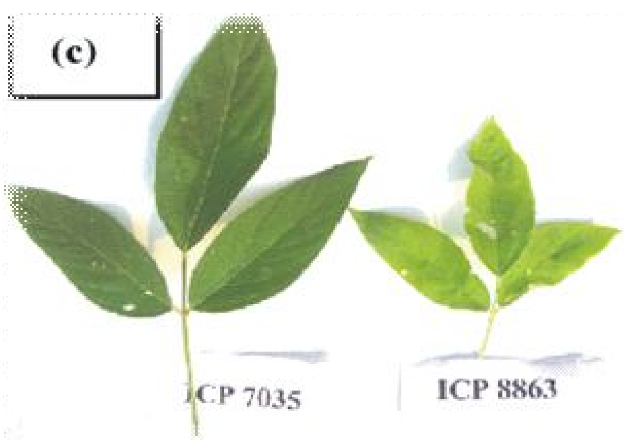


**Plate 1(c) :** Uninfected resistant (ICP 7035) and susceptible (ICP 8863) checks showing dark green leathery leaves and light green non-leathery leaves, respectively.

thick cuticle in resistant genotypes hinders mite penetration, preventing SMD pathogen transmission.

To confirm the association of dark green, leathery leaves with SMD resistance, grafting experiments can be conducted, as demonstrated by Kumar *et al.* (2005). In summary, our findings imply that the leathery texture and leaf thickness in resistant plants become more apparent in later crop growth stages. Quantitative methods for measuring leaf colour and thickness will enhance precise resistant plant selection. Our results support the notion that Pigeonpea Sterility Mosaic Virus (PPSMV) infects crops early on, with resistance possibly linked to mites' inability to feed on resistant plants. Further confirmation through replicated experiments with $F_2$ derived advanced progenies will validate the association of leaf traits with SMD resistance.

## Conclusion

Our study highlights the complex nature of Sterility Mosaic Disease (SMD) resistance in pigeonpea, suggesting that the number of genes controlling resistance may vary depending on the parental material. Notably, one hybrid (ICPL 20325 × Hy3C) from the class of moderately resistant × moderately resistant displayed robust resistance to SMD with a Disease Severity Index (PDI) of 9.09, indicating the incorporation of SMD resistance genes from both parent plants. Conversely, the crosses involving BRG 3 as one of the parents exhibited monogenic resistance, primarily governed by the BRG-3 alleles.

The potential link between dark green, leathery leaves and SMD resistance is a promising avenue for further investigation. Grafting experiments using advanced progenies from the mentioned crosses could provide valuable insights and confirm this association. If verified, this finding may revolutionize the breeding strategies for SMD-resistant pigeonpea lines and varieties. It could enable indirect selection for specific leaf traits in segregating generations, ultimately contributing to the overall improvement of pigeonpea crops.

This study sheds light on the genetic complexities and potential mechanisms underlying SMD resistance in pigeonpea, paving the way for more targeted breeding programs and the development of more robust SMD-resistant cultivars.

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