Inheritance Studies of Sterility Mosaic Disease (SMD) Resistance Cross Gullyal white×BSMR736 in Pigeonpea (*Cajanus cajan* (L.) Millsp.)

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Plant Gene and Trait, 2014, Vol.5, No.4 doi: 10.5376/pgt.2014.05.0004

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Abstract Sterility mosaic disease (SMD) is a major problem of pigeonpea all over India and particularly in Karnataka, Maharashtra and parts of Andhra Pradesh. Pigeonpea yields have been declining due to heavy and recurring occurrence of the SMD in southern Karnataka and some districts of northern Karnataka. The occurrence of this disease in farmer’s field is reported to vary between 0 and 100%. A comprehensive study of variability in the sterility mosaic pathogen has revealed the occurrence of five different isolates of the pigeonpea sterility mosaic virus in India. With this view in the background, the objective was undertaken in the present study. The nature of inheritance of SMD in resistant (BSMR736) and susceptible (Gullyal white) genotypes. SMD incidence observed in parents, Reaction of F2:3 families of Gullyal white×BSMR736 cross. Out of 300 F2:3 families field evaluated, 55 showed resistant phenotype, 209 were moderately resistant and 36 families were susceptible for PPSMV. A basic knowledge of inheritance and number of genes governing the traits are essential for efficient selection. The ratio of F2:3 families to SMD were corroborated with respectively F2 individual plant. cross Gullyal white×BSMR736 and governed by two genes designated as $SV_1$ and $SV_2$. The dominant allele of one gene ($SV_1$) has inhibitory action on the character (resistance) govern by other ($SV_2$) gene. Based on these, the presence of dominant allele of $SV_1$ gene in one locus suppresses the action of dominant allele of $SV_2$ (resistance) gene present on another locus resulting in susceptible phenotype.

Keywords Sterility mosaic disease; Pigeonpea sterility mosaic virus; *Cajanus cajan*; Inheritance

Background Food legumes are important component of modern agriculture in meeting nutritional requirement of human beings. They are also a critical component of large and small animal feeds. Pigeonpea [*Cajanus cajan* (L.) Millsp.] is a major grain legume crop of the semi-arid tropics, which is cultivated on 2.65 million tones, with an annual production of over 4.33 million tones contributing to about 5% of the total world production of pulses (FAOSTAT 2012; http://faostat.fao.org). Nearly 90% of the global pigeonpea cultivation is confined to India and Nepal, the remainder is in Africa (6%), the Caribbean (2%) and other Southeast Asian countries (N. Maxted and S. Kell., 2012). In India, the major pigeonpea growing states are Maharashtra, Uttar Pradesh, Karnataka, Madhya Pradesh, Andhra Pradesh and Gujarat.

It is a major source of protein to about 20% of the world population (Wanjari et al., 2003) and is an abundant source of minerals and vitamins (Saxena et al., 2002). It is an ideal supplement to cereal and tuber-based diets of resource constrained farmers. It comes well in marginal lands and it is popular among small and marginal farmers in the Indian subcontinent. The productivity of pigeonpea crop is very low in India with 799 kg/ha as compared to its productivity potential of 2000 kg/ha. Factors such as lack of improved cultivars, poor crop husbandry, pests and diseases are responsible for this. Wild relatives have now been reported to possess many agronomically important traits such as resistance to pests and diseases (Reddy et al., 1995; Sharma et al., 1984), salinity tolerance (Subbarao et al., 1991) and high protein content (Saxena et al., 1996), all of which would be useful in cultivated pigeonpea. Over an hundred fungal, bacterial and viral pathogens are known to attack pigeonpea crop in the world (Nene et al., 1989). Major diseases include *Fusarium wilt*
caused by *Fusarium udum* Butler, sterility mosaic disease (SMD) caused by pigeonpea sterility mosaic virus (PPSMV) and Phytophthora blight by *Phytophthora drechslerii* and pests such as pod borer (*Helicoverpa armigera*), maruca (*Maruca vitrata*), pod fly (*Melanagromyza obtusa*), plume moth (*Exelastis atomosa*) cause substantial reduction to pigeonpea crop. The pigeonpea sterility mosaic virus (PSMV) is transmitted and perpetuated through a mite, *Aceria cajani* (Nene et al., 1977).

Sterility Mosaic disease (SMD) occurs with regularity under suitable conditions, spreads rapidly, leading to epidemics where in the yield losses depend on the growth stage at which infection occurs (Ganapathy et al., 2011). The affected plants look are green with excessive vegetative growth without any flowers and pod, hence, it is also referred to as green-plague: under congenial conditions it is known to spread rapidly like a *plague*, leading to severe epidemics. Generally, an early infection results in a 95%–100% loss in yield (Reddy et al., 1990; Kulkarni et al., 2003), on the other hand, losses from late infection depend on the level of infection ranging from 26%–97% (Kannaiyan et al., 1984). Maximum virus incidence and yield losses occur in ratooned and perennial pigeonpea.

Srinivas et al. (1997) reported the inheritance of resistance and allelic relationship in three resistant pigeonpea sources for strain 2 of sterility mosaic pathogen. The inheritance of resistance has been determined by crossing resistant genotypes ICP 7035, ICP 7349 and ICP 8850 with susceptible genotypes BDN 1 and LRG 30 and used to obtain information on their allelic relationship. Parents, F1 and F2 generations screened using “infector hedge row technique” and indicated the dominance of resistance in certain crosses and the dominance of susceptibility in others. Amala Balu and Rathnasamy (2003) studied the pattern of inheritance of the sterility mosaic resistance in pigeonpea, where the two susceptible parents, Prabath and Co 5, two resistant parents, ICPL 83024 and ICPL 83027 and their F1 hybrids and F2 progenies were screened for sterility mosaic disease. The study reported the moderately resistant to SMD in F1s while, in F2 the rating scale for SMD ranged from 3 to 9. The F2 generation of the four combinations fitted well with the segregating ratio of 13:3 for susceptibility and resistance indicating non-allelic interaction of two factors.

1 Results

1.1 Development of F2 and F2:3

Crossing of the parental genotypes and raising of mapping population were carried out under nylon net to avoid cross pollination through insect pollinators. Gullyal white, a highly susceptible cultivar to SMD was crossed with a resistant parent, BSMR736 and the resultant F1s was raised. The F2 seeds collected from a single F1 plant which was confirmed for true hybridity using DNA markers were used to plant over 300 F2 plants. All the 300 F2 plants were selfed to obtain the F2:3 families that were used for phenotyping the incidence of SMD at Agricultural Research Station (ARS), Bidar. ARS, Bidar is known for its endemism for the occurrence of PSMD, hence called ‘hot spot’ of SMD. The hedge row coupled with artificial inoculation of diseased leaves together with the mites was successfully used in manifesting the PSMD. Sowing of ICP 8863, a susceptible variety, was done about 4 weeks prior to the sowing of actual experimental material. Over 10-15 days old hedge border rows and infector rows infected with PPSMV through the stapling of diseased leaves could successfully manifest the disease symptoms in more than 80 per cent of the plants (Figure 1).
In leaf stapling techniques, one diseased leaflet per primary leaf was stapled (Figure 2). As the stapled leaflets from the infected plants get dried, mites from the infected leaves migrate to healthy leaf and inoculates the virus. The PPSMV susceptible genotype

![Figure 2: Pigeonpea plants are stapling with SMD infected leaf samples](image)

ICP 8863, used to developed infector hedge rows on the border of the experimental field was found ideal genotype in bring up the disease and the pathogen and mites multiplication in sufficient threshold on the hedge and successfully served as source of PPSMV inoculums. Generally, the mites are carried through wind onto the test rows in the field, which was evident by the observed symptoms on all susceptible progenies in the field.

1.2 Inheritance study for PPSMV resistance in pigeonpea

Reactions of $F_{2:3}$ families were classified into different categories following the PSMD reaction scale (Singh et al., 2003), wherein, the disease score of 0 to 10% was taken as resistant, 10.1% to 30% as moderately resistant and 30.1% to 100% as susceptible. The disease reaction of families was classified into above said categories and they were further prospected for various known genetic rations and their test of fit using Chi-square test. Finally, the $F_{2:3}$ families showing moderate resistant or susceptible phenotype to PPSMV were combined together into susceptible category where, the observed segregation ratio of 245:55 (susceptible: resistant) in Gullyal white×BSMR 736 cross was recorded. In this cross, the families with susceptible reaction were more in number than the families with resistant phenotype. The segregation pattern in $F_{2:3}$ families were comparable to the 13 (susceptible):3 (resistant) ratio. Based on the observed segregation ratio it was suggestive that the PPSMV resistance is under two gene control with non-allelic interaction of the type ‘inhibitory gene interaction’. The goodness of fit for expected and observed values as tested by Chi-square test is presented in the (Table 1). The mean disease reaction of individual families was recorded and per cent disease was computed. Reaction of $F_{2:3}$ families of Gullyal white×BSMR 736 cross is presented in the (Table 2). Out of 300 $F_{2:3}$ families field evaluated, 55 showed resistant phenotype, 209 were moderately resistant and 36 families were susceptible for PPSMV. A basic knowledge of inheritance and number of genes governing the traits are essential for efficient selection. The ratio of $F_{2:3}$ families to SMD were corroborated with respectively $F_2$ individual plant. The goodness of fit to mendelian and gene analysis was done and segregation of resistant and susceptible plant tested by Chi-square analysis with (n−1) degrees of freedom, where ‘n’ was the total number of segregating classes (Stansfield,1986).

2 Discussion

A few published reports do indicate that susceptibility to be under the influence of recessive genes (Murugesan et al., 1997). Further, reaction of pigeonpea to SMD is not only dependent on the

| No. of $F_{2:3}$ families | Total | $\chi^2$ (Cal) | $\chi^2$ (Tab) | Ratio |
|--------------------------|-------|---------------|---------------|-------|
| Observed                 | 55.00 | 245.00        | 300           | 0.032 | 3.80  | 13:3 |
| Expected                 | 56.25 | 243.75        | 300           |       |       |      |

Note: Disease scoring and classification was according to Singh et al. (2003)
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Table 2 Distribution of F2:3 families of the cross Gullyal white x BSMR 736 in the PSMD score ranges of resistant, moderately resistant and susceptible based on disease incidence in SMD sick garden at ARS Bidar

| Sl. No. | Per cent PSMD disease incidence | Phenotype          | No. of F2:3 families |
|---------|--------------------------------|--------------------|---------------------|
| 1       | 1 to 10% of plant infected      | Resistant          | 55                  |
| 2       | 10.1 to 30% of plants infected  | Moderately resistant| 209                 |
| 3       | 31.1 to 100% of plants infected | Susceptible        | 36                  |
| Total   |                                 |                    | 300                 |

Resistance source of host plant, but also on the PPSMV isolate dominating in the region. Srinivas et al. (1997) studied the inheritance of resistance for isolate of SMD and observed that resistance was dominant. Hence, source of resistance and the allelic composition of recessive parent influence the inheritance of PPSMV resistance. Singh et al. (1983) conducted sick plot study with three susceptible, Pant A2, UPAS 120 and T 21 and five resistant lines, Pant A3, TCP 3783, TCP 6997, TCP 7035 and ICP 7119 and crossed between them which also pointed at the possible existence of different resistance sources and non-allelic interactions.

The majority of families were grouped into moderately resistant and susceptible class and only a few families were found to be resistant type. Based on these observation, we hypothesis that the susceptibility is controlled by a dominant gene and the resistance is under the control of recessive gene. Since the susceptibility is controlled by dominant genes, number of plant with high level of resistance to SMD is fewer in F2:3 generation. Studies have reported that the genetics of SMD resistance is greatly influenced by many factors, such as the resistance source used, SMD isolates and methodology adopted for scoring, indicating complex nature of it (Saxena, 2008).

It was suggestive from the results that the PPSMV resistance was governed by two genes designated as SV1 and SV2 (Table3). Singh et al. (1983) the dominant allele of one gene (SV1) has inhibitory action on the character (resistance) govern by other (SV2) gene. Based on these assumption, 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. Only those plants with recessive allele of SV1 gene and dominant allele of SV2 gene might have shown resistant phenotype. On the basis of this hypothesis we propose that the following types of genotypes might be possible for resistance and susceptible phenotypes in plants.

3 Materials and Methods

The PPSMV resistant variety-BSMR 736 and susceptible variety-Gullyal white were obtained from Agricultural Research Station (ARS), Gulbarga and Karnataka to develop suitable segregating generations and recombinant inbred line population. BSMR736 variety released from Agricultural Research Station, Badnapur, Maharashtra, is known to be consistently and stably resistant to PPSMV (Saxena et al., 2008) Gullyal white, a highly preferred variety for its dhal quality is also highly susceptible for PPSMV infection. These lines were maintained by continuous selfing for

Table 3 Types of genotypes might be possible for resistance and susceptible phenotypes in plants

| SMD Genotypes       | F2 ratio | SMD Phenotypes | F2 epistatic ratio |
|---------------------|----------|----------------|-------------------|
| (SV1-SV2)           | 9        | Susceptible    |                   |
| (SV1-SV2sv2)        | 3        | Susceptible    | 13 (susceptible):3 (resistant) |
| (sv1sv1SV2)         | 3        | Resistant      |                   |
| (sv1sv1sv2sv2)      | 1        | Susceptible    |                   |
several generations in the pigeonpea breeding programme at Agricultural Research Station, Gulbarga.

The individual flowers of the female parent, Gullyal white, were hand emasculated and pollinated with the pollen dust from the male parent, BSMR736, in the cool hours of the day to get sufficient F1 seeds. The F1 plants along with their parents were grown and true F1 plants were selfed by covering nylon net to prevent out crossing through honey bees and other insect pollinators. The F1 plants were raised and the mature seeds were collected and advanced to F2 generation. A set of 300 individual F2 plants from Gullyal white×BSMR 736 cross were grown in the field under the nylon net at Main Agricultural Research Station (MARS), Dharwad. The plot chosen for this experiment was positioned to have sugarcane field on one side and rice field on the other side, both known to favors the manifestation of SMD in pigeonpea.

Before actual sowing of F2:3 families, ‘infector-hedge - rows’ of susceptible variety ICP8863 were established. Briefly, an infector-hedge consisting of two widely spaced rows of the susceptible cultivar, ICP8863 was sown one month prior to actual planting experiment. The susceptible check was sown in regular intervals of test sample, which would serve as spreader of disease on the field. The F2:3 families were sown in two replications, where at least 30~40 plants per replication was maintained. A spacing of 60 cm between the rows and 30 cm between plants within a row was maintained with recommended package of practices without any control measures for SMD.

3.1 Artificial inoculation
The PPSMV artificial inoculation in the field was done according to “leaf stapling” and “infector-hedge” techniques (Nene and Reddy, 1976). The PPSMV infected pigeonpea leaves along with mites were collected from farmers’ fields of Afzalpur village near Bidar and used for artificial inoculation. One diseased leaflet per primary leaf was generally used. The diseased leaflet was folded on the primary leaf in such a way that its lower surface came into contact with the primary leaf of the test seedlings. As the stapled leaflets from the infected plants get dried, mites from the infected leaves migrate to healthy leaf and inoculates the virus.

3.2 Ratooning
The ratooned and perennial pigeonpea is known to have highest SMD incidence. In case of late infection of PPSMV, symptoms may not occur even if the genotype is susceptible to PPSMV, however, when plants are ratooned symptoms appear prominently on the new growth (Kumar et al., 2002; Jones et al., 2004). Ratooning is known to favor the manifestation of SMD in pigeonpea. The experimental F2:3 families of present study were cut from first branch position from ground and irrigated to encourage the sprouting. After 30 days of ratooning, the disease incidence was scored. The ratios of F2:3 to SMD was grouped the following the standard scale (Singh et al., 2003) as given below: 0~10% of plants infected: Resistant; 10.1%~30% of plants infected: Moderately resistant; 30.1%~100% of plants infected: Susceptible.

Authors’ Contributions
Shivarudrappa B Bhairappanavar conducted the study. B Fakrudin analyzed and interpreted the data. Shivarudrappa B Bhairappanavar wrote the manuscript. B Fakrudin designed the study by assistance. K.R.S.Sambasiva Rao reviewed the manuscript.

Acknowledgments
This research work was supported by Indian Council of Agricultural Research (ICAR) for the financial support (AKI-PGI) and Department of Biotechnology (DBT), Ministry of Science and Technology, Government of India (DBT Programme Support Project). We thank the Project Monitoring and Mentoring Committee (PMMC) members, Dr. V. P. Gupta, Dr. N. Seetharama; Dr. P. Balasubramaniam, Dr. M. B. Chetti, Dr P.U. Krishnraj and Dr. Y.D.Narayana for their helpful suggestions from time to time. We acknowledge the timely help of Dr.C.R.Konda, in facilitating disease studies at Bidar.

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