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Biochemical Basis of Resistance to Sterility Mosaic Disease in Pigeon Pea Genotypes

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ABSTRACT

Background: Sterility mosaic is the most destructive disease of pigeon pea causing yield losses up to 95 per cent. Pigeon pea genotypes are found to vary in different biochemical characteristics, thereby playing a crucial role in resistance to sterility mosaic disease. The best approach to manage the disease is by adopting resistant genotypes for cultivation, which is highly necessary in the areas with high sterility mosaic incidence.

Methods: Field screening of 41 pigeon pea genotypes was conducted during *Kharif* 2021 and 2022 at the Indian Institute of Pulse Research, Regional Research Center, Dharwad, to identify the resistance sources to *Pigeon pea sterility mosaic virus*. All the test genotypes were artificially inoculated by leaf stapling technique at 15-20 days after sowing and the infector hedge row technique was also employed to develop high disease pressure. Observation on per cent disease incidence was recorded at the peak period of the disease. The study also focused on biochemical components that confer resistance in pigeon pea. Further, the leaf samples were collected from the selected pigeon pea genotypes showing resistance and susceptible reactions and statistically analysed for total phenol content, peroxidase and polyphenol oxidase activity. The genotypes showing resistance and susceptible reactions were analysed for leaf chlorophyll content using SPAD Meter.

Result: Seven genotypes were found resistant to sterility mosaic disease with a mean disease incidence of 1.9-8.86 per cent. SPAD chlorophyll meter reading revealed that chlorophyll content remained high in all resistant/moderately resistant genotypes as compared to susceptible genotypes. The resistant genotypes were shown to have higher phenol content, peroxidase and polyphenol oxidase activity in comparison to susceptible genotypes suggesting their crucial role in imparting resistance.

Key words: Disease reaction, Pigeon pea, Polyphenol oxidase, SPAD Chlorophyll meter reading, Sterility mosaic disease.

INTRODUCTION

Pigeon pea [*Cajanus cajan* (L.) Millsp.] is an important pulse crop of India with >75 per cent contribution to the world's pigeon pea production. It is a substantial source of protein for the human diet. It is a perennial shrub with the chromosomal number 2n = 2x = 22 grown annually for food, feed, fodder or fuel throughout Asia, Africa, Latin America and the Caribbean. Globally, pigeon pea is cultivated in over 6 million hectares, with a production of 5.47 million tonnes (FAO, 2022). India is leading in pigeon pea production with an area of 4.54 million hectares and annual production of 3.83 million tonnes (Anonymous, 2021).

Sterility mosaic disease which is caused by *Pigeon pea Sterility Mosaic Virus* (*PPSMV*), is considered as an economically important disease of pigeon pea in India and *Aceria cajani* Channabasavanna transmits the virus in a semi-persistent manner (Manjunatha *et al*., 2012). Based on their molecular characterization, *PPSMV* - I has five negative sense dsRNA segments whereas, *PPSMV* - II has more than six negative sense dsRNA segments. Based on the morphological and molecular characteristics, both *PPSMV* - I and *PPSMV* - II viruses are placed in the family *Fimoviridae* and under the genus *Emaravirus* (Kumar *et al*., 2017).

The field symptoms of SMD (Sterility Mosaic Disease) include bushy plants, mild mosaic, severe mosaic, smaller leaves, excessive vegetative growth, stunted development, chlorotic ring patches, partial sterility and complete sterility (Siril *et al*., 2022). Early infection can cause 95 to 100 per cent yield loss, but late infection loses vary depending on the level of infection and ranges from 26 to 100 per cent

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(Dharmaraj *et al*., 2004). This is a major area of concern as pigeon pea demand in India is constantly increasing. It is worthwhile to study pigeon pea genotypes that are resistant to sterility mosaic disease and the mechanisms underlying their resistance, since cultivating resistant genotypes is an efficient and affordable alternative to chemically controlling plant diseases. Host plant resistance may be genetic or ecological. A possible defense mechanism against mite vectors and viruses is morphological and phenological formation of secondary metabolites, anatomical features, nutritional status and biochemical and microbiological characteristics (Manjunatha *et al*., 2021).

It is generally known that the disease resistance mechanism is a complicated process and that plants create a variety of biochemical responses in response to the invasion of a disease-causing organism. The current study focuses on identifying SMD-resistant pigeon pea genotypes by screening under field conditions and biochemical components which aids in defining the features that play a major role in SMD resistance in pigeon pea.

MATERIALS AND METHODS

Screening of pigeon pea genotypes for resistance to sterility mosaic disease

A large number of genotypes were screened at AICRP on Pigeon pea, Voluntary Center, RARS, Vijayapur during *Kharif* 2020. A total of 41 pigeon pea genotypes were collected from AICRP on Pigeon pea, Voluntary Center, RARS, Vijayapur and ICAR-IIPR, RRC, Dharwad. Further, the genotypes were subjected to field screening at Indian Institute of Pulse Research, Regional Research Center, Dharwad during *Kharif* 2021 and 2022 against Sterility Mosaic Disease (SMD). These were sown in rows of 4 m in length with a spacing of 90 × 20 cm with ICP 8863 and Gulyal Local as susceptible checks (infector row) and Bahar as a resistant check in a non-replicated augmented design. All the recommended agronomic practices were followed, except disease management practices. All the test seedlings of pigeon pea genotypes were artificially inoculated by leaf stapling technique as per Nene *et al*. (1981) at 15-20 days after sowing and infector hedge row technique was also employed to develop high disease pressure. Per cent disease incidence (PDI) was calculated by using the following formula and the genotypes were categorized into different disease reactions based on average PDI by adopting the criterion followed in All India Co-ordinated Research Project (AICRP) on pigeon pea.

$$\text{Per cent disease incidence} = \frac{\text{Number of diseased plants}}{\text{Total number of plants observed}} \times 100$$

Scale adopted for categorizing pigeon pea genotypes against SMD.

Disease incidence (%)	Reactions
0-10	Resistant
11-30	Moderately resistant
>30	Susceptible

Biochemical analysis of SMD resistant and susceptible pigeon pea genotypes

The biochemical parameters were assessed for healthy plants at 15 DAS and diseased plants at 45 DAS to know the biochemical activity before and after the infection of the virus. The leaf samples collected from the selected pigeon pea genotypes showing both resistant and susceptible reactions and were analysed for chlorophyll content using SPAD Meter (60 and 90 DAS), total phenol content, peroxidase and poly phenol oxidase activity. Selected genotypes *viz*., Bahar (Resistant), KA 17-2 (Resistant), BDN 2013-45 (Moderately resistant) and ICP 8863, Gulyal local and IPA 15-02 (Susceptible) genotypes were taken for phenol and enzyme activity assay. Analysis of variance (ANOVA) was carried out for different parameters by following a Completely Randomized Design with three replications using IBM SPSS statistics 21. Estimation of phenols was carried out with the Folin-Ciocalteu reagent by following the method of Bray and Thorpe (1954). The peroxidase activity from the leaves was assayed by the method of Kumar and Khan (1982) and the polyphenol oxidase activity was assayed spectrophotometrically by the procedure given by Mayer *et al.* (1965).

RESULTS AND DISCUSSION

The most practical and economically advantageous method of controlling SMD is the production and cultivation of resistant/tolerant cultivars of the crop. In the present study, seven genotypes among 41 showed resistant reactions with a mean SMD incidence ranging between 1.9 - 8.86 per cent. KA 17-2 (3.70%), IPA 19-26 (1.9%), BDN 711 (3.8%), MAL 52 (5.56%), BAUPP 17-14 (5.58%), IPA 14-4A (6.67%) and MAL 53 (8.86%) were the pigeon pea genotypes found to be resistant to SMD (Table 1). The study showed that 14 genotypes were moderately resistant and 20 genotypes were susceptible to SMD. Among susceptible genotypes, IPA 15-06 recorded the maximum SMD incidence (95.24%). Similarly, susceptible checks ICP 8863 and Gulyal local showed SMD incidence of 96.3 per cent and 100 per cent, respectively and resistant check Bahar recorded the least incidence of 1.8 per cent (Fig 1). Similar screening studies by Raghavendra (2003), concluded that ICP 8863, TTB 7, TS 3R, BRG 2, HY 3C and locally cultivated cultivars such a Gulyal local, Benur local have a high incidence of sterility mosaic disease with mild to severe mosaic symptoms. In the multilocational screening trials, genotypes ICPL-16078, ICPL-16086 and ICPL-16087 displayed resistance reactions (Sayiprathap, 2022). SMD prevalence reached 100% in several cultivars, completely ruining the harvest and causing severe mosaic symptoms.

Biochemical analysis of SMD-resistant and susceptible pigeon pea genotypes

Plant pathogens induce changes in the normal metabolism of the host following infection. Similarly, some biochemical changes in the tissues are brought by viral infection in

plants. As the infection progresses, the host develops several defense mechanisms. Initial defensive reactions include various antimicrobial substances in host plants,

Table 1: Per cent disease incidence and disease reaction of pigeon pea genotypes to sterility mosaic disease.

Genotype	Per cent disease incidence	Disease reaction
AKTM 1637	55.56*	S
AL 2207	70.59	S
BAUPP 17-14	5.58	R
BDN 2013-2	29.17	MR
BDN 2013-45	18.75	MR
CRG 16-04	60.61	S
DA 19-1	41.94	S
DA 2020-1	22.86	MR
GRG 622	82.76	S
ICPIL 17124	28.57	MR
IPA 14-4A	6.67	R
IPA 15-02	31.20	S
IPA 15-06	95.24	S
SKNP 1823	70.83	S
PAU 881	85.7	S
AL 2324	86.8	S
Pusa Arhar 21-29	79.5	S
CRG 16-01	90	S
IPAE 15-05	29.7	MR
CRG 16-12	18.9	MR
BDN 711	3.8	R
IPA 15-19	25.71	MR
IPA 19-55	16.67	MR
IPA 7-2	13.79	MR
KA 17-2	3.70	R
KA 18-2	28.0	MR
MAL 52	5.56	R
PA 509	34.29	S
PT 10-1-1-2	15.63	MR
PT 11-4-4-1	29.41	MR
PA 634	65.63	S
PUSA 992	58.82	S
RVSA 28-1-1	78.57	S
SKNP 1701	92.59	S
TDRG 59	12.00	MR
IPA 19-26	1.9	R
PUSA 197	73.6	S
MAL 53	8.86	R
BDN 716	43.3	S
BSMR 736	16.7	MR
Susceptible check-ICP 8863	96.3	S
Susceptible check-Gulyal local	100	S
Resistant check-Bahar	1.8	R

*Mean Per cent disease incidence of 2021 and 2022; R- Resistant, MR- Moderately Resistant, S- Susceptible.

Table 2: Total phenol content, peroxidase activity and polyphenol oxidase activity in SMD susceptible and resistant pigeon pea genotypes.

Genotype/Variety	Total phenols (mg/g)			Peroxidase content ($\Delta A\ min^{-1}\ g^{-1}$)			Polyphenol oxidase (PPO) ($\Delta A\ min^{-1}\ g^{-1}$)			SPAD chlorophyll meter reading (SCMR) SCMR mg/g FW	
	15 DAS (Healthy)	45 DAS	Per cent increase over healthy	15 DAS (Healthy)	45 DAS	Per cent increase over healthy	15 DAS (Healthy)	45 DAS	Per cent increase over healthy	60 DAS	90 DAS
KA 17-2 (R)	2.07	3.60	42.50	3.88	4.92	21.14	0.43	1.10	60.91	45.45	46.65
Bahar (R)	2.10	3.55	40.85	3.67	4.75	22.74	0.46	1.24	62.90	46.50	48.20
BDN 2013-45 (MR)	1.87	2.75	32.00	3.30	3.94	16.24	0.44	0.77	42.86	44.50	42.50
ICP 8863 (S)	1.62	1.95	16.92	2.19	2.30	4.78	0.25	0.37	32.43	43.60	36.20
Gulyal local (S)	0.94	1.10	14.55	2.40	2.60	7.69	0.38	0.54	29.63	46.20	34.55
IPA 15-06 (S)	1.85	2.15	13.95	2.28	2.44	6.56	0.36	0.50	28.00	43.40	36.50
SE.m. ±	0.04	0.08	-	0.05	0.04	-	0.07	0.06	-	2.80	2.67
CD at 1%	0.14	0.27	-	0.17	0.14	-	0.16	0.13	-	1.02	0.92
CV (%)	3.28	4.20	-	2.34	4.34	-	2.80	3.09	-	4.45	4.77

R- Resistant, MR- Moderately resistant, S- Susceptible.

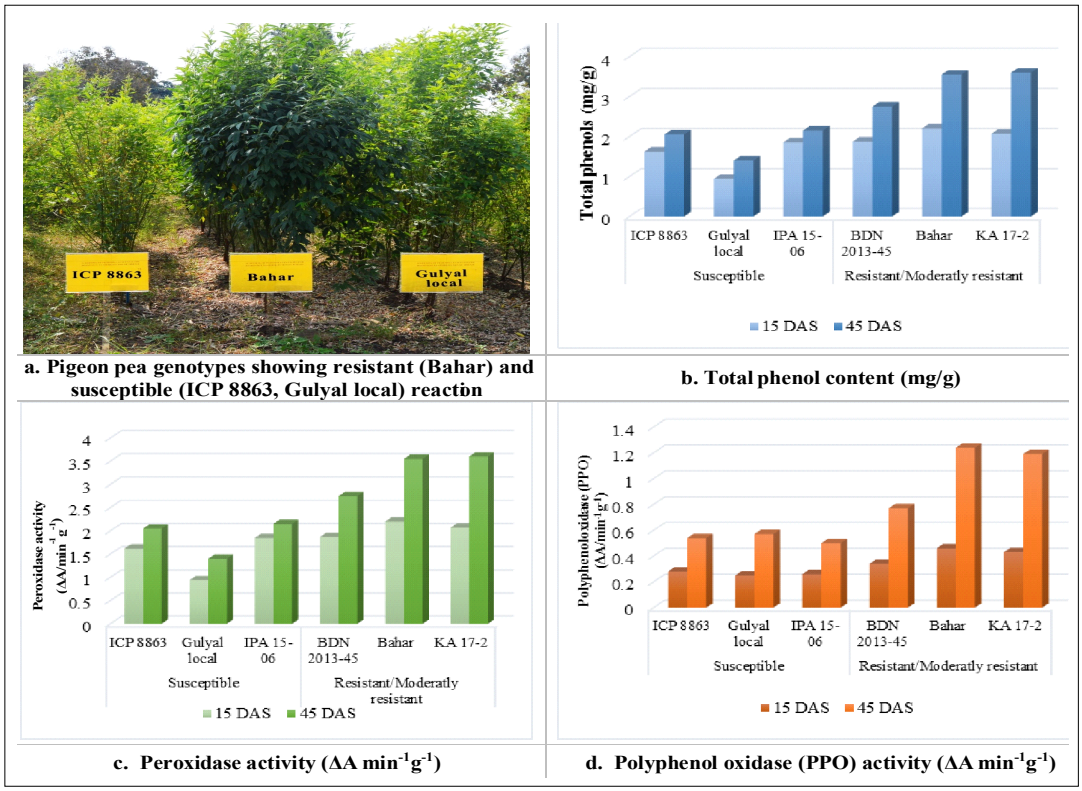
including phenols and defense enzymes (Osbourn, 1996). The present investigation focused on the study of some biochemical elements of SMD-resistant and susceptible pigeon pea genotypes to understand the effect of sterility mosaic disease and its role in resistance reactions.

Total phenol content

In the current investigation, resistant genotypes, Bahar (3.55 mg/g) and KA 17-2 (3.60 mg/g) recorded a higher amount of phenolic content, followed by moderately resistant genotypes BDN 2013-45 (2.75 mg/g) and IPA 15-06 (2.15 mg/g), ICP 8863 (1.95 mg/g) and Gulyal local (1.10 mg/g) genotypes at 45 DAS (Table 2 and Fig 1). A higher per cent increase of phenol content over healthy was recorded in the resistant lines than in the moderately or susceptible genotypes. Resistant and moderately resistant genotypes showed a range of 32.00-42.50 per cent increase over the healthy, whereas it was 13.95-16.92 per cent increase over the healthy sample in the case of susceptible lines. Per cent increase of phenol content over healthy was found in KA 17-2 and Bahar with 42.50 and 40.85, respectively. The high phenol content in resistant genotypes might be explained by the presence of more sugar content, which serves as a precursor for the production of phenolic compounds. In susceptible varieties, phenol content and its increase during disease progression were the least. This agrees with the findings of Rathi (1986). Total phenolic content of healthy leaves of resistant/ moderately resistant varieties, ICP 7035 (4.416 mg) and BRG 1 (2.981 mg), exhibited slightly higher phenolic content than the corresponding leaves of susceptible varieties, Maruti (2.241 mg) and TTB 7 (2.251 mg) in a study conducted by (Manjunatha *et al*., 2012).

Peroxidase (PO) activity

Peroxidase (PO) is one of the first enzymes responding and providing fast defense against plant pathogens. An increase in the activity of peroxidase is observed in all the genotypes infected by sterility mosaic disease (Table 2 and Fig 1). But resistant and moderately resistant lines showed a high per cent increase of peroxidase activity over healthy (16.24 - 22.74) compared to the susceptible lines (4.78-6.56). The highest enzymatic activity was recorded in the resistant genotypes, KA 17-2 (4.92 ΔA min^{-1}g^{-1}) and Bahar (4.75 ΔA min^{-1}g^{-1}) at 45 days after sowing compared to the susceptible genotypes *viz*., PA 15-06 (2.44 ΔA min^{-1}g^{-1}), ICP 8863 (2.30 ΔA min^{-1}g^{-1}) and Gulyal local (2.60 ΔA min^{-1}g^{-1}). Moderately resistant line, BDN 2013-45 recorded PO activity of 3.94 ΔA min^{-1}g^{-1} fresh weight. The least PO activity was recorded in the healthy sample of ICP 8863 (2.19 ΔA min^{-1}g^{-1}) and IPA 15-06 (2.28 ΔA min^{-1}g^{-1}). These results agree with the findings of Sudharani (2016). Less peroxidase activity in susceptible genotypes indicated that PO was suppressed leading to the weakening of defense mechanisms against *Pigeon pea sterility mosaic virus*.



a. Pigeon pea genotypes showing resistant (Bahar) and susceptible (ICP 8863, Gulyal local) reaction

b. Total phenol content (mg/g)

c. Peroxidase activity (ΔA min^{-1}g^{-1})

d. Polyphenol oxidase (PPO) activity (ΔA min^{-1}g^{-1})

Fig 1: Fig showing a) Resistant and susceptible pigeon pea genotypes, b) Total phenol content (mg/g), c) Peroxidase activity (ΔA min^{-1}g^{-1}) and d) Polyphenol oxidase (PPO) activity (ΔA min^{-1}g^{-1}) of some SMD susceptible and resistant pigeon pea genotypes.

Polyphenol oxidase (PPO) activity

Polyphenol oxidase (PPO) is an important in the initial stage of plant defense where membrane damage causes release of phenols such as chlorogenic acid. In the present study, it was found that infection of *PPSMV* resulted an increase in PPO activity in all the pigeon pea genotypes analysed, but susceptible lines showed small change only (Table 2 and Fig 1). At 45 days after sowing, resistant and moderately resistant varieties such as KA 17-2, Bahar and BDN 2013-45 exhibited higher PPO activity (1.1, 1.24 and 0.77 ΔA min^{-1} g^{-1} respectively) than that in the susceptible varieties, ICP 8863, Gulyal Local and IPA 15-06 (0.54, 0.37 and 0.50 ΔA min^{-1} g^{-1}, respectively). The least PPO activity was recorded in the healthy sample of ICP 8863 (0.54 ΔA min^{-1} g^{-1}) and IPA 15-06 (0.50 ΔA min^{-1} g^{-1}). PPO activity was increased from 15 DAS to 45 DAS in all the genotypes, but resistant genotypes showed a higher per cent increase of PPO activity over healthy compared to susceptible genotypes. Resistant genotypes exhibited higher per cent increase of PPO activity over healthy sample which ranged from 42.86-60.91 compared to susceptible genotypes that showed 28.00-32.43 per cent increase over healthy sample. These results are in conformity with reports of Anuradha *et al*. (2015). Upon infection with SMD, the only appreciable change recorded was the activity of PPO in susceptible variety where it increased considerably in the resistant genotypes.

SPAD chlorophyll meter reading (SCMR)

SPAD chlorophyll meter (SCMR) showed that chlorophyll content increased slightly in resistant genotypes after infection while it was decreased in all other genotypes (Table 2). SPAD chlorophyll meter readings (SCMR) of different pigeon pea genotypes recorded at 60 DAS and 90 DAS is presented in the Table 2. The highest SCMR was recorded in Bahar with 48.20, but the least was recorded in the susceptible genotype Gulyal local with 34.55. The highest drop SPAD readings was recorded in susceptible genotype compared to the moderately resistant genotype. Reduction trend was more in susceptible variety than moderately resistant varieties while it was increased in resistant genotypes. Similar conclusions on decline of chlorophyll concentration following pathogen infection were made by Arora *et al.* (2009) and Sudharani (2016).

CONCLUSION

One of the key control approaches for reducing viral disease losses is host plant resistance. This technique of management is reasonably affordable, easy to use and "ecologically sound" for farmers. Seven of the 41 pigeon pea genotypes screened were identified to be resistant to the sterility mosaic disease. Resistant genotypes include KA 17-2, IPA 19-26, BDN 711, MAL 52, BAUPP 17-14, IPA 14-4A and MAL 53. Chlorophyll content remained high in all resistant/moderately resistant genotypes as compared to susceptible genotypes. Resistant genotypes were shown to have higher phenol content, peroxidase and polyphenol oxidase activity. Although phenol content, PO and PPO activity increased after infection in all genotypes, but the per cent increase over healthy was observed to be higher in resistant and moderately resistant genotypes. High phenolic compounds and higher activity of peroxidase and polyphenol oxidase enzymes in leaf tissue of pigeon pea appeared to be important biochemical constituents in imparting resistance to SMD. These biochemical parameters can be used as markers for screening genotypes for *PPSMV*.

Conflict of interest

The authors declare that there is no conflict of interest in preparing this manuscript.

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