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Biochemical Alterations in Different Pigeon Pea Genotypes due to Sterility Mosaic Disease

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ABSTRACT

Background: The sterility mosaic disease in pigeon pea caused by pigeon pea sterility mosaic virus is a serious threat to pigeon pea cultivation in India. A little information is available on the biochemical changes in pigeon pea plant against the SMD disease. Hence present study was taken to know the biochemical alterations in different pigeon pea genotypes due to sterility mosaic disease.

Methods: Studies were conducted on the composition of the chlorophyll pigment, total soluble protein (%), total sugars, phenol, tannins, peroxidase and phenol peroxidase in six different genotypes.

Result: The results of the study showed that there was a reduction in chlorophyll pigment composition, total soluble protein (%) and total sugars in all infected genotypes, but the reduction in resistant genotypes was less compared to other genotypes. In resistant genotypes, the amounts of phenols, tannins, peroxidase and phenol peroxidase were increased, whereas in susceptible genotypes, they were roughly equal to the amounts in uninoculated genotypes. The increase in phenols, tannins, peroxidase and phenol peroxidase in resistant genotypes can be correlated with disease resistance.

Key words: Pigeon pea, Protein, Resistant, Susceptible, Uninoculated.

INTRODUCTION

Pigeon pea is the second most important legume crop after chickpea. It is a member of the Fabaceae family and the genus *Cajanus*, according to (Ghadge *et al.,* 2008). It is one of the most significant legume crops grown in a variety of agro-ecological systems in arid and semi-arid climates. It is the world's sixth most important pulse crop, with India contributing more than 70% of the entire production (Jorrin *et al*., 2021). Fusarium wilt and sterility mosaic disease are two diseases that cause severe yield losses in India (Kaushik *et al*., 2013).

The sterility mosaic disease of pigeon pea caused by the pigeon pea sterility mosaic virus (PPSMV) is an economically important disease, resulting in annual losses of about 300 million US$ annually in India (Reddy *et al*., 1998). The pigeon pea sterility mosaic virus belongs to the genus of Emaravirus (Elbeaino *et al*., 2014). This disease is transmitted by the eriophyid mite (*Aceria cajani*, Channabasavanna) in a semi-persistent manner (Seth, 1962; Kulakarni *et al.,* 2002). The typical symptoms of SMD infected plants includes yellow mosaic, chlorotic spots, reduced leaf size, excessive vegetative growth and the infected plants become partial or complete sterile (Jones *et al*., 2004).

The use of resistant cultivars in the management of sterility mosaic disease is the safest, most economically viable option. However, it was challenging to build in the broad-based resistance into the plants due to the prevalence of several location specific strains. Therefore, development of resistant cultivars is most important in order to protect the crop from the disease threat. There is a limited information on the biochemical changes in pigeon pea plants against the SMD disease. Hence, the current study was done on the quantitatively estimate the levels of chlorophyll content, soluble protein content, total sugar content, total phenol content, total tannin content and the activities of the enzymes peroxidase and phenol peroxidase in different genotypes of pigeon pea.

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MATERIALS AND METHODS

All the experiments were conducted at the Department of Agriculture entomology, Tamil Nadu Agriculture University. The genotypes included in the present study were selected after initial screening against the SMD disease.

Plant material

In this investigation a total of eight genotypes were selected for this study based on the percent disease incidence (Sharma *et al*., 2015). These ge notypes were categorized

into resistant (CO7, LRG-52) having ≤10.0% incidence); moderately resistant (BRG-4, CO-9 having 10.1-20.0% incidence); susceptible (C0-8, LRG-105 having 20.1-40.0% incidence) and highly susceptible (ICP- 8863, VBN-3 >40.0% incidence) based upon the initial screening studies.

SMD inoculum maintenance

The sterility mosaic disease and mite colony were maintained on the susceptible cultivar - (ICP-8863 Shree Maruti), which was grown in pots of (25 × 25 cm) pots. At two leaf stage the disease was infected by stapling the SMD infected leaves.

Virus transmission

The selected genotypes were planted in the pots of (25 × 25 cm) pots at the two leaf stage and all these genotypes were infected with disease by stapling the infected leaves. After 30 days after inoculation of disease the physiologically active leaves were selected and packed in polythene bags brought back to laboratory for biochemical analyses.

Chlorophyll pigment composition

About 250 mg of leaf was taken and macerated with 80% of acetone and the samples were centrifuge at 3000 rpm for 10 minutes and then supernatants were collected and made up to the volume of 25 ml. Measurements on Chlorophyll 'a', 'b' and total chlorophyll were done at different wavelengths according to the protocol (Yoshida *et al.,* 1971).

Total soluble protein content

About 250 mg of the leaf sample was macerated in phosphate buffer and then centrifuged at 3000 rpm for 10 minutes and supernatants were collected and made up to 25 ml. Then 5 ml alkaline copper tartarate reagent (ACT) with 0.5 ml of folin ciocalteau reagent will be added to the test tube. The development of colour was observed after 30 mins and OD was measured at 660 nm according the protocol of (Lowry *et al.*, 1951).

Total sugars

The total sugars were estimated by using the anthrone method of Hedge and Hofreiter (1962). A fresh leaf sample (100 mg) was taken and boiled in the hot water bath and 2.5 N HCL (5 ml) was added. The leaf sample was boiled for 3 h in order to hydrolyse the sample. After cooling down, it was neutralized by adding sodium carbonate until effervesce stopped. Make the volume to 100 ml and centrifuge. The supernatant (1.0 ml) was collected and boiled for 8 minutes and then anthrone (4.0 ml) reagent was added. The content will be allowed to cool till the colour changes from green to dark green. The OD value will be measured at 630 nm.

Total phenols

The total phenol content was estimated according to the procedure of Malik and Singh, (1980). Approximately 250 mg of leaf sample was macerated in 10 ml of 80% ethanol and the extract content was boiled in hot water for 10 minutes, allowed to cool and then centrifuged at 5000 rpm for 10 minutes. The supernatant was collected and made up to 25 ml. One ml of supernatant will be added with 2 ml of 20% sodium carbonate and followed by 1 ml of folin reagent. The OD value was measured at 660 nm after colour development.

Total tannins

The total tannin content was estimated according to the procedure (Price *et al.,* 1978). The 100 mg dried leaf samples were taken and ground in methonal. After 24 hours, it was centrifuged at and 1 ml of supernatant was collected. To this 5 ml of vanillin hydrochloride reagent was added and readings were taken at 500 nm after 20 min.

Peroxidase

The enzyme peroxidase content was estimated according to the procedure to Peru (1962). The leaf sample (500 mg) was macerated in 10 ml of phosphate buffer and centrifuged at 5000 rpm for 15 min and then to the 1 ml of supernatant, 3 ml of pyrogallol was added. Then, 0.5 ml of H_2O_2 will be added to the supernatant and the OD value was measured at 430 nm for 2 minutes at every 30 second interval.

Phenol peroxidase

The polyphenol oxidase enzyme content was done according to procedure (Galeazzi and Sgarbieri, 1981). The leaf sample (500 mg) was macerated with 10 ml of phosphate buffer solution and centrifuged at 5000 rpm for 15 min and to the 1 ml of supernatant, 1ml of buffer solution and 1 ml of catechol were added. Then the volume was made up to 10 ml and the OD value was measured immediately at 420 nm for 2 min at 30 second interval.

The data collected from all experiments was subjected to two-way analysis of variance (ANOVA). The means were compared for significance using Tukey's HSD test and the values presented are mean of three replicates ± standard error (SE).

Results and Discussion

Disease response in genotypes

The SMD infected genotypes displayed a wide range of symptoms depending upon their genetic makeup. The resistant genotypes (CO-7, LRG-52) showed modest mosaic symptoms, chlorotic ring spots, with no reduction in the leaf size in contrast to highly susceptible genotypes (VBN-3, ICP-8863). The moderate resistant (Co-9, BRG-4) and susceptible genotypes (CO-8, LRG-105) showed a range of symptoms between severe mosaic and chlorotic ring spot symptoms on the leaves.

Chlorophyll content

The decrease in chlorophyll content in diseased plants compared to uninoculated plants has been documented in many cases (Lobato *et al.,* 2010; Shakeel *et al.,* 2016). There was a reduction in chlorophyll content in all infected genotypes compared to the un inoculated genotypes. It was

found that the resistant genotypes C07 and LRG-52 had the highest levels of chlorophyll a, b and total chlorophyll (Fig 1a, b,c). This was followed by the moderately resistant genotypes BRG-4 and CO9 and then by susceptible genotypes like CO8 and LRG-105. The highly susceptible genotype VBN-3, ICP-8863, had the least chlorophyll.

The SMD infected plants showed a decrease in chlorophyll content compared to uninoculated plants, as reported by Narayana Swamy and Ramakrishnan, (1965). Sinha and Srivastava (2010) and Ananthu and Umamaheswaran (2019) reported similar results in mungbean infected with mung bean yellow mosaic virus and ginger infected with virus.

Protein percentage

Many proteins involved in disease resistance have been noticed in many cases (Carvalho *et al*., 2006). The soluble protein content in different genotypes was expressed as percentage of protein. It was discovered that infected susceptible genotypes had a greater reduction in protein content than the resistant genotypes.

The highly susceptible genotypes VBN-3 and ICP-8863had the greatest reduction in protein content compared to the un inoculated ones followed by susceptible genotypes (C0-8, LRG-105). Protein levels in resistant [CO (Rg)-7] and moderately resistant (Co-9 and LRG-105) genotypes did not differ significantly (Fig 1d). Similar results reported by Chatterjee and Ghosh (2008) in Mestha against yellow vein mosaic disease. Rajinimala *et al*. (2009) and Anuradha *et al*., (2015) also discovered similar results in bitter gourd against yellow mosaic virus, in banana against bunchy top virus.

Total sugars estimation

Viral infection alters the carbohydrate synthesis in infected plants. The considerable reduction in chlorophyll content has negative impacts on carbohydrate synthesis. It was found that the reduction in sugar content in resistant genotypes over the uninoculated ones was less compared to the infected highly susceptible genotypes. The maximum reduction of total sugar content over the uninoculated was found in the highly susceptible genotypes VBN-3 and ICP-8863, followed by susceptible genotypes (C0-8, LRG-105) (Fig 1e). A relatively minimum reduction in total sugar content was observed in resistant (CO7 and LRG-52) and moderately resistant genotypes (CO-9 and BRG-4). Similar results were obtained in Cucumber against cucumber mosaic virus, Ginger due to virus infection (Shalitin and Wolf, 2000; Ananthu and Umamaheswaran, 2019).

Total phenol estimation

The phenols generally contribute to resistance in plants by synthesizing the lignin and suberin involved in the formation of physical barriers (Singh *et al.,* 2014). The increase in phenol content was observed in the genotypes Co (Rg)-7, LRG-105 and CO-9 (Fig 1e). In the rest of all genotypes, it was found that there was a decrease in the phenol content. The increase in phenols was correlated with high disease resistance. The possible reason for the decrease in the phenol content in susceptible genotypes was due to the suppression of plant defence mechanisms. Cotton genotypes infected with the cotton leaf curl Burewala virus and mungbean genotypes infected with phytoplasma and mungbean yellow mosaic virus yielded similar results (Siddequi *et al.,* 2014; Hameed *et al.,* 2017; Madhumitha *et al.,* 2020).

Total tannin estimation

Tannins are active secondary metabolites involved in plant chemical defence against the invasion of pathogens. It was discovered that infected resistant genotypes had greater increase in tannin content than the uninoculated ones, whereas susceptible genotypes increase in tannin content was comparatively less than the uninoculated ones. The tannin content was highest in the resistant group and it was significantly different in moderately resistant, susceptible and highly susceptible genotypes. This higher accumulation of tannins was found in the genotypes LRG-52 and CO-7 (Fig 1f) and was correlated with high disease resistance. The lower tannin content in the genotypes ICP-8863 and VBN-3 was due to high susceptible reactions. Similar results of an increase in tannin content were found in ground nuts against leaf spot and rosette disease, tomato genotypes against early blight (Mohammed *et al.,* 2019; Medić-Pap *et al.,* 2015).

The peroxidase enzyme estimation

Peroxidase is one of the enzymes providing fast defence against invading plant pathogens (Sulman *et al.,* 2001). It was discovered that infected resistant genotypes had a greater increase in peroxidase content than in uninoculated genotypes, whereas susceptible genotypes had an increase in peroxidase content that was roughly equal to uninoculated ones. The highest enzyme activity was correlated with disease resistance and low activity was correlated with susceptibility. The resistant genotypes (Co(Rg)-7, LRG-105, had the most activity, while the moderately resistant genotypes (Co-9, BRG-4) had the least. In susceptible genotypes (LRG-105, CO8) and highly susceptible genotypes (ICP-8863, VBN-3), the peroxidase activity is more or less similar to the uninoculated ones (Fig 1h). Similarly, increased peroxidase enzyme activity was found in resistant cotton genotypes infected by Burewala virus and mung bean genotypes against phytoplasma (Siddequi *et al.,* 2014; Hameed *et al.,* 2017).

The phenol peroxidase enzyme estimation

PPO catalyzes the oxidation of phenols to free radicals, thus creating an unfavourable environment for pathogen development (Mohamed *et al.,* 2012). It was found that the amount of phenol peroxidase in infected, resistant genotypes was higher than in uninoculated genotypes. However, the increase in phenol peroxidase content in susceptible genotypes (C0-8 and LRG-105) and highly susceptible genotypes (ICP-8863 and VBN-3) and in highly susceptible genotypes (ICP-8863 and VBN-3) was similar to that in uninoculated genotypes (Fig 1i).

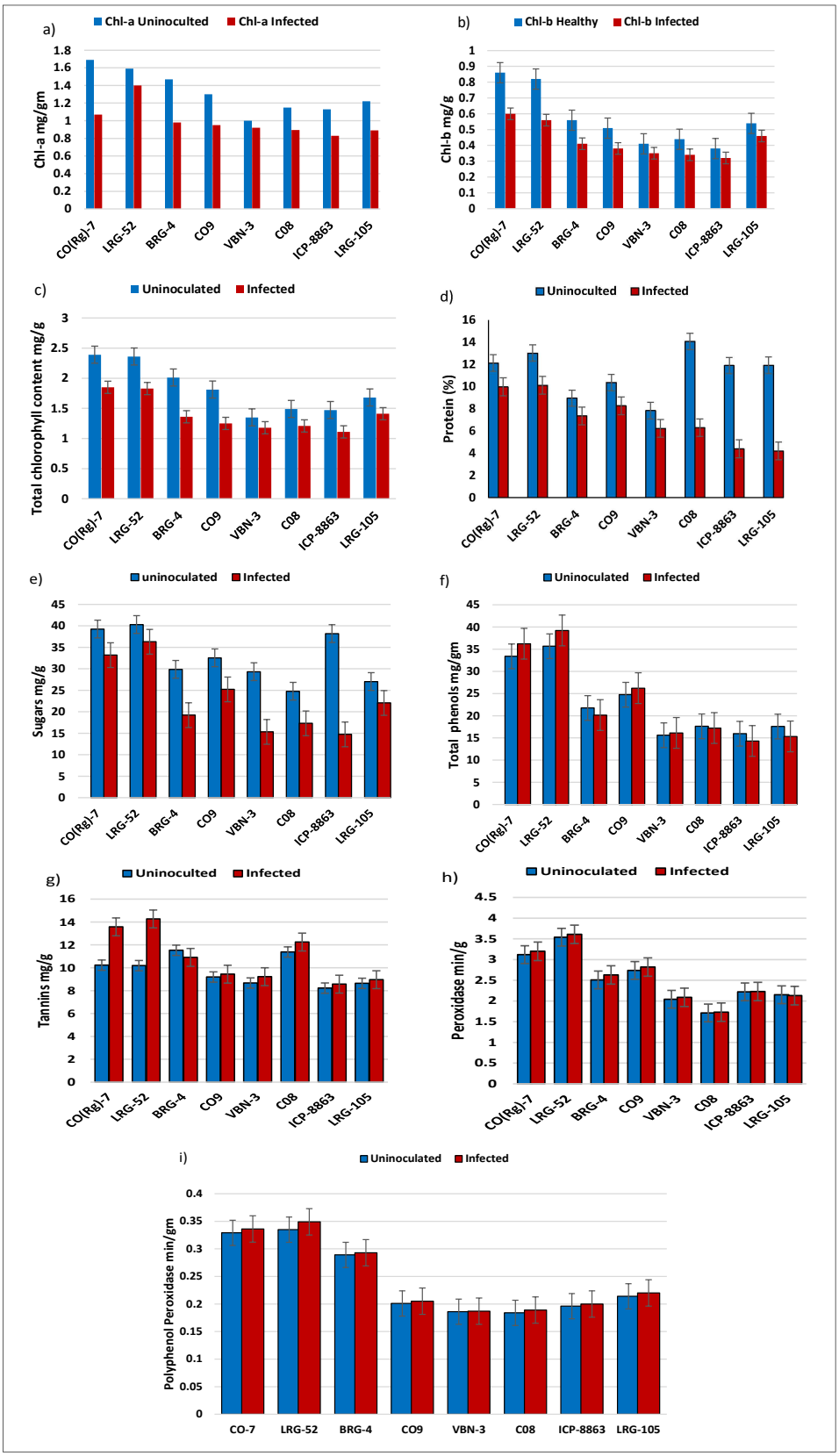


Fig 1: a) chlorophyll a content mg/g; b)- Chlorophyll b content mg/g; c)- Total chlorophyll content mg/g; d)- Protein (%); e)- Total sugars mg/g; f)- Phenols mg/g; g)- Tannins mg/g; h)- Peroxidase min/g; i)- Phenol peroxidase min/g.

A slight increase in the phenol peroxidase activity was noticed in the moderatly resistant genotypes (BRG-4 and C0-9). Similarly, increased activity of phenol peroxidase enzymes in resistant cotton genotypes infected by the Burewala virus and mung bean genotypes against phytoplasma (Siddequi *et al.,* 2014; Hameed *et al.,* 2017).

Conclusion

In this study there was a significant difference between resistant and susceptible genotypes in terms of biochemical and antioxidant enzymes. The anti-nutritional factors phenols, tannins and antioxidant enzymes such as peroxidase and phenol peroxidase play a role in disease resistance. All the infected genotypes have reduced contents of the chlorophyll pigments, protein content and total sugars content. The phenols, tannins, peroxidase and phenol peroxidase activities were high in resistant genotypes compared to susceptible genotypes, indicating they play a significant role in disease resistance.

Conflict of interest

All authors declared that there is no conflict of interest."

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