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Effect of sterility mosaic disease on the physiological and biochemical aspects of pigeonpea

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Abstract
Biotic and abiotic stress causes many physiological and biochemical alterations in plants. Some alterations result from stress on the plants, and some are related to the plant's response to stress. Pigeonpea sterility mosaic disease is one of the major constraints to the production of pigeon pea, an important pulse of India. This disease is endemic to the Indian subcontinent. In this report, the various biochemical parameters of PPSMV-infected and healthy plants were evaluated.

Keywords: Proline, chlorophyll, catalase, virus

Introduction
Viral infection alters various physiological and biochemical parameters of the host. It alters and siphons various metabolites for its replication and dissemination. It has been reported that plant viruses reduce the photosynthetic rates of their hosts (Magyarosy *et al*., 1973; Rahoutei *et al*., 2000) [8, 13] and lower photosynthesis by reducing electron transport rates in infected plants at the photosystem II (PSII) level. (Balachandran *et al*., 1997; Balachandran and Osmond, 1994) [1, 2]. In tobbaco mosaic virus (TMV), disturbances of the chlorophyll fluorescence parameters has been observed before symptom development indicating that there is an impact of the virus on PSII function. (Balachandran *et al*., 1994) [3]. TMV-infected plants accumulate Coat protein in chloroplast that affects the functioning of PSII complex (Banerjee *et al*., 1995; Reinero and Beachy, 1986) [4, 14].
At the same time, upon CMV (cucumber mosaic Virus) infection in tobacco, the polypeptide proteins of OEC (oxygen evolving complex) is greatly reduced (Lindbeck *et al*., 1991) [7]. Similarly, one of the Cucumber mosaic virus encoded movement protein has been shown to affects sugar metabolism and transport in tobacco and melon plants (Mauck *et al*., 2014; Shalitin *et al*., 2002) [9, 15].
CMV infected plants have also been shown to have higher free amino acids but lower total soluble sugar content. The Salicylic acid content was higher in infected leaves whereas Jasmonic acid content were reported to be similar in both infected and uninfected leaves (Mauck *et al*., 2014) [9]. Further, in Turnip crinkle**.**
Tobacco mosaic virus (TMV) 126 kDa replication protein has been shown to disrupt Auxin signaling (Padmanabhan *et al*., 2006) [11].
Rice dwarf virus (RDV) induces stunting and leaf darkening, symptoms that are charac- teristic of GA-deficient rice mutants. An interaction be-tween the RDV outer capsid P2 protein and the rice ent-kaurene oxidase has been identified (Collum and Culver, 2016) [5].
Pigeonpea Sterility Mosaic Virus are associated with sterility mosaic disease (SMD) of pigeonpea. The SMD of pigeonpea was first reported in 1931 from the Bihar in India (Patil and Kumar, 2015) [12]. However, the genome sequences of the emaraviruses associated with this disease has been recently published (Elbeaino *et al*., 2014) [6]. SMD is mostly endemic to Indian subcontinent countries in Asia (Bangladesh, Nepal, Thailand, Myanmar, Sri Lanka and cause a heavy loss to pigeonpea as it is one of the major pulse crops of Asia.
The genomes of PPSMV are segmented with each virion having 4-6 segments. At the same time, some PPSMV variant harbor 5 genomes in its virion (Elbeaino *et al*., 2014) [6]. The proteins encoded by these genome segment has not been characterized yet. Further, there is limited report

on impact of PPSMV infection on the physiology of Pigeonpea crops. In this paper, effect of PPSMV infection on the physiological parameter of pigeonpea has been studied.

Material and Methods

Chlorophyll and Carotenoid Content

The quantity of chlorophyll and carotenoid in the pigeon pea leaves (Rajendra Arahar 1) was determined using the method proposed by Hiscox and Israelstam (1979) [16] and DMSO (dimethyl sulfoxide). A 500 mg leaf sample was initially placed in a test tube containing DMSO (4 mL). Subsequently, the test tubes were placed in an oven at 60 °C for around 4 hours to enhance pigment extraction. The test tube was then removed and allowed to cool to room temperature, after which absorbance was measured using a spectrophotometer at 645 nm, 663 nm and 470 nm. Dimethyl sulfoxide, DMSO was used to represent blank.

Catalase Activity

The activity of catalase was determined as described by (Mitsuda and Yasumatsu, 1955) [10]. 500 mg of Peageon pea leaves (Rajendra Arahar 1) was homogenised with 2 ml phosphate buffer (pH 6.0, 0.1 M) centrifuged at 10000 rpm for 10 minutes at 4 °C. Pellet was discarded and the supernatant was diluted to 4 mL with phosphate buffer. In a test tube, 1 mL of a 1 percent H_2O_2 solution, 0.5 mL of enzyme extract (homogenate) and 2.5 mL phosphate buffer were added and mixed and then incubated for 3 minutes at 30 °C. The reaction was then ended by adding 2% H_2SO_4 (1 mL). The reaction mixture's H_2O_2 residue was then titrated with 0.01 N $KMnO_4$ solution to a faint pink tint that lasted at least 15 seconds. In the same way, a blank was made by replacing the enzyme extract with water and absorbance was taken at 405 nm.

Proline Content

1 gm of infected leaf was crushed in liquid nitrogen using a pestle motor. After crushing the leaf into powder, it was placed in a test tube and ethanol (70%) was added and incubated at 4°C overnight. Later, it was centrifuged for 5 minutes at 12000 RPM at 4 °C. The supernatant was diluted to 5ml and 1 ml was taken in a fresh tube to which 2 ml reaction mixture (ninhydrin 1% (w/v) in acetic acid 60% (v/v), ethanol 20% (v/v) was added. The tube was heated for approximately 20 minutes at 95 °C in a water bath after sealing. After that, the test tube was removed and allowed to cool to room temperature before the absorbance was measured using a spectrophotometer at 520 nm.

Results and Discussion

Pigeonpea (Rajendra Arahar 1) infected and healthy plants were collected from regions of Bihar. As shown in figure 1, there is loss of chlorophyll and mild mosaic symptoms can also be observed in infected leaves. Further, these leaves were used for various biochemical assay. The healthy leaves were used as negative control.

Fig 1: PPSMV infected Pigeon pea crop collected from Bihar. The infected leaves show chlorosis and mosaic symptoms as compared to healthy plants

Total Chlorophyll content: The total chlorophyll content of infected and healthy leaves was extracted as described in method section and absorbance were taken at 663nm. As observed from Figure 2, the infected leaves have lower absorbance as compared to healthy leaves suggesting that the amount of chlorophyll is lower in infected leaves as compared to healthy leaves. The result is in accordance of the symptoms observed in Figure 1. As can be observed from symptoms that infected leaves have undergone chlorophyll loss thereby yellowing of leaves has occurred.

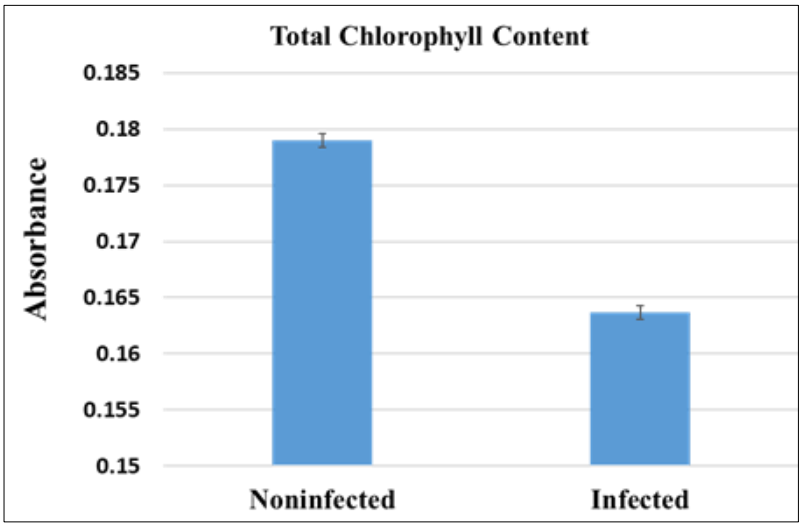

Fig 2: Total chlorophyll content of infected and healthy leaves. The chlorophyll was extracted as described in method section. The absorbance of the resulting solution was recorded at wavelength 663 nm

Carotenoid content of infected and uninfected leaves

Carotenoid pigments are accessory pigments in plants. It acts as an antenna molecule to siphon the light energy towards reaction center and at the same time it can also act as a photo protective agent by dissipating extra light energy as heat. Therefore, Carotenoid also protect plants from abiotic stress like photoinhibition.

Hence, it was of interest to investigate, the effect of PPSMV infection on carotenoid content of pigeon pea leaves. As evident from Figure 3, the absorbance of the sample having carotenoid extracted from leaves was reduced by 15% in infected sample as compared to healthy leaves indicating that there is loss of chlorophyll and carotenoid that would hamper the photosynthetic process.

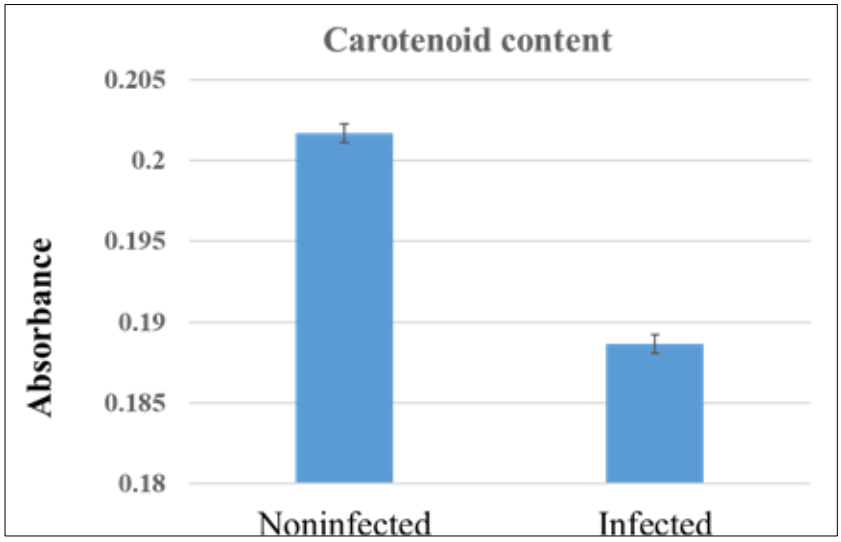

Fig 3: Carotenoid content was measured by taking absorbance at 470 nm of infected and uninfected leaves

Proline content: The proline content in cell is directly correlated to the severity of abiotic stress. Therefore, it was of interest to find out the effect of PPSMV infection on the proline content in pigeonpea. As evident from figure 4, based on the absorbance it can be said that the proline content of PPSMV infected leaves were drastically reduced as compared to healthy leaves. As the PPSMV leaves show chlorosis, it may be that upon PPSMV infection, there is loss of nitrogen. Hence, the content of non-essential amino acid like proline has reduced significantly. However, the result needs further verification and investigation.

Catalase activity of infected and uninfected leaves

Upon viral infection there are reports that the amount of Reactive oxygen species (ROS) increases in order to defend virus. So, in an attempt to find out the effect of PPSMV infection on ROS scavenger production, catalase assay was performed. As shown in figure 4, the catalase activity was lowered during PPSMV infection, thereby resulting in higher amount of ROS which might be involved in host defense mechanism. However, to come to a conclusion further investigation is required.

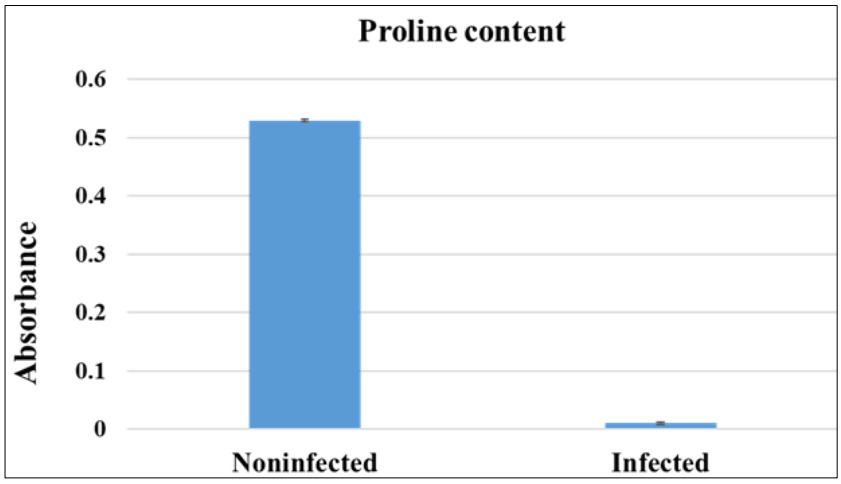

Fig 4: Proline content of infected and uninfected leaves

Discussion

Interactions between virus and plant affect the crop production by reducing the yield. Plant virus alters the primary and secondary metabolites production as well as their partitioning. It also affects the enzymatic activity of antioxidant enzymes in the plants. They also alter the production and partitioning of photosynthetic assimilates.

PPSMV is one of the most important plant viruses that cause a heavy economic loss in Bihar. Therefore, it was of interest to understand the phytochemical changes that occur upon virus infection in pigeon pea. As it has been reported for various

viruses that they alter the photosynthetic pathway especially PSII, so it was of interest to find out the effect of viral infection on photosynthesis of pigeonpea. As shown in Figure 2, the amount of chlorophyll is reduced upon virus infection. However, the reduction is not very significant indicating that it might be involved in reduction in yield by rendering the plant sterile. As explained earlier that this viral infection sometimes is named as "green plague".

However, as evident from Figure 3, the carotenoid content was reduced by 15%, suggesting that there is significant loss of accessory pigments. However, it is not understood the reason behind the loss of carotenoid. Hence, this result needs further investigation.

Apart from photosynthesis, virus infection alters amino acid metabolism. Therefore, to this end the proline content of infected and uninfected leaves were measured spectrophotometrically. As evident from figure 4, the proline content was significantly reduced upon viral infection. It is one of the most significant result of this study. However, it needs further investigation to understand the mechanism leading reduction in proline. At the same time in some literature, it has been reported that free amino acid content increases upon viral infection but here we see a large reduction in proline content.

Plant virus also alter the antioxidant enzymes. In this study, the catalase amount present in infected and healthy leaves were measured through spectrophotometric analysis. It was found that the Catalase content was reduced in infected leaves leading to the formation of higher amount of ROS. The ROS is directly related host defense toward infection. ROS provide immunity to viral infection and is involved in hypersensitive response.

Therefore, the study shows that upon PPSMV infection, the photosynthesis rate might get reduced and ROS increases to provide immunity to pigeon pea against PPSMV.

Conclusion

Viral infections significantly impact the physiology of host plants, as evidenced by alterations in photosynthetic processes, amino acid metabolism, and antioxidant enzyme activity. The study on Pigeonpea Sterility Mosaic Virus (PPSMV) infection in pigeon pea plants reveals a reduction in chlorophyll and carotenoid content, potentially hindering photosynthesis. Moreover, a substantial decrease in proline content suggests a disturbance in nitrogen balance, while reduced catalase activity indicates heightened levels of reactive oxygen species (ROS), possibly as part of the plant's defense mechanism. These findings underscore the intricate interactions between viruses and plants, leading to compromised crop yield. Further research is warranted to elucidate the mechanisms underlying these physiological changes and their implications for crop management strategies in combating viral infections. Understanding the intricate dynamics of virus-plant interactions is crucial for devising effective strategies to mitigate the economic losses associated with such infections.

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