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Biochemical basis of resistance in papaya genotypes systemically infected with *Papaya ringspot virus*

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Abstract

Papaya ringspot disease caused by Papaya ringspot virus (PRSV) has been identified as a serious threat to papaya in Indian subcontinent. Symptoms of disease include mosaic, shoestring and ringspot symptoms on leaves. Fruits also become disfigured with ringspot symptoms. However little is known about the plant virus interaction. In the present investigation, phenol, total soluble protein content, activities of defense related enzymes like peroxidase, polyphenol oxidase and phenylalanine ammonia-lyase were quantitatively estimated in the leaves of resistant (Pusa Nanha) and highly susceptible (local cultivar) genotypes of papaya (Carica papaya L.). The results revealed a significant increase in amount of phenol, protein and defense related enzymes in inoculated plants of resistant genotype, indicating their positive correlation with disease resistance, thus this can be considered as a biochemical marker for studying host pathogen interactions.

Keywords- Papaya ringspot virus; Carica papaya L.; host pathogen interactions; resistant; susceptible genotypes

I. INTRODUCTION

Papaya (*Carica papaya* L.), Family *Caricaceae*, is an important fruit crop both in tropics and subtropics, because of its high nutritional and medicinal value. In India, among different fruit crops grown, papaya cultivation ranks fifth with regards to area and production. Cultivation is challenged by many diseases especially Papaya ring spot disease caused by *Papaya ringspot virus* (PRSV). Infection was reported to occur in every region of the country where papaya is grown irrespective of the agroclimatic conditions and the disease can result in crop losses of 85 to 90% [9 and 10].

The use of resistant varieties being the safest, economical, and effective option to manage plant viruses, many papaya varieties have been evolved over the years. But new strains of PRSV could overcome the resistance of host plant. The present investigation was conducted on the quantitative estimations of phenolic compounds, protein contents and activities of defense related enzymes, indicating their role in virus inoculated and uninoculated plants of resistant and susceptible genotypes.

II. MATERIALS AND METHODS

A. Plant material

Seeds of resistant cultivar, Pusa Nanha was obtained from IARI, Pusa, New Delhi and susceptible local cultivar from Instructional Farm, College of Agriculture, Vellayani. Seeds were sown in pots (30 cm diameter) containing potting mixture of sand, soil and cow dung (1:1:1). The pots were maintained in insect-proof glasshouse.

B. Sources of viral inoculum

The inoculum of virus was obtained from the artificially inoculated plants of a highly susceptible local cultivar, maintained in an insect proof glasshouse.

C. Virus transmission and identification

For the purpose of virus inoculation, infected leaves of the susceptible local cultivar were homogenized in 0.1 *M* potassium phosphate buffer (1:1.5 w/v) (pH 7.0). The homogenate was squeezed through cotton wool and used as standard inoculum. The papaya plants at six leaf stage were mechanically inoculated on the upper surface of the leaves with the standard inoculum, using carborundum powder, 600 mesh as an abrasive. Ten, two week old plants of each genotype were

inoculated and the culture was maintained in insect proof glasshouse. Data were collected on the time required for first symptom appearance and type of symptoms developed up to 60 days of inoculation. A same second set was left uninoculated to serve as control. The presence of virus was confirmed through serological and molecular techniques.

D. Estimation of biochemical constituents

The contents of phenol [4], total soluble protein [3], activities of peroxidase [19], polyphenol oxidase [12] and phenylalanine ammonia-lyase [6] were estimated, for the leaves harvested from both genotypes at 5, 10, 15, 30 and 60 days after inoculation (DAI). Leaf samples from control plants were also analysed.

E. Statistical analysis

The data collected from all the experiments were analysed separately for each parameter and subjected to one way analysis of variance (ANOVA) in Completely Randomised Design using computer programme Excel.

III. RESULTS AND DISUSSION

3.1. Disease response

PRSV inoculated plants, of both the genotypes showed a wide range of symptoms depending on their genetic makeup.

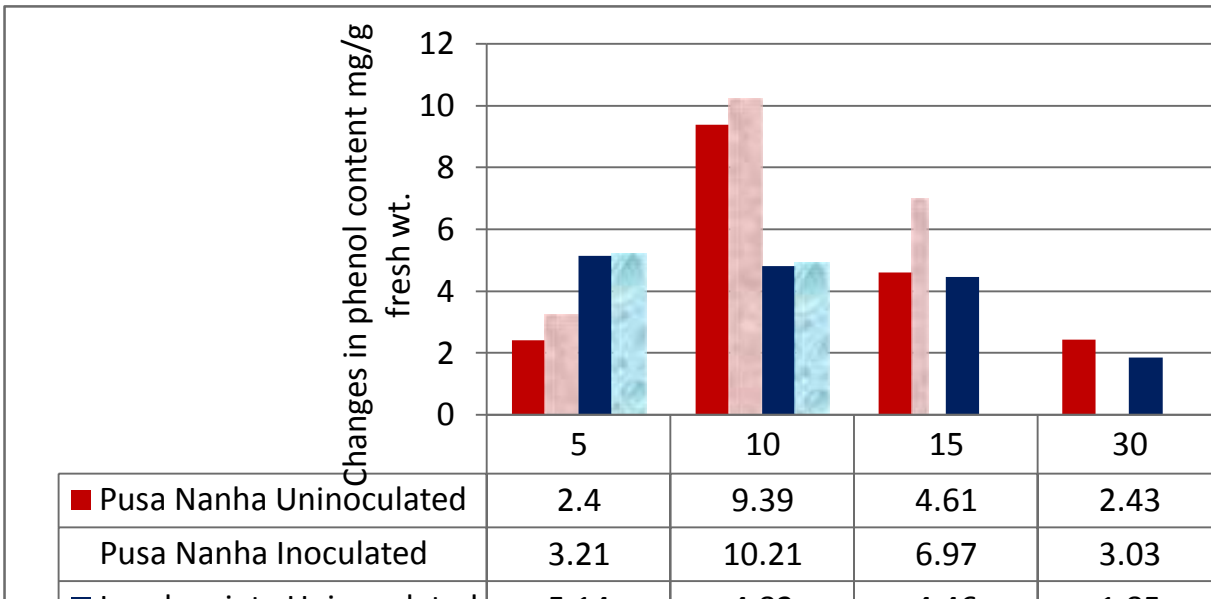
Susceptible local variety initiated disease symptoms as water soaked oily vein clearing symptoms, on the younger leaves, 14 days post inoculation. Most prominent and characteristic diagnostic symptom noticed was the development of shoe string symptoms on the leaves, were in the leaf lamina was highly reduced and narrowed down, resembling a shoe string. The mosaic symptom was also observed, sometimes localised or covering the complete leaf area. Leaves also got puckered, with dark green raised areas demarcated by veins seen either localised or affecting the complete leaves. Complete or incomplete concentric rings and numerous chlorotic spots were observed on infected leaves and fruits, hence the name *Papaya ringspot* disease. Infected leaf became completely distorted and disfigured, with marked size reduction.

All the mechanically inoculated plants of resistant genotype, Pusa Nanha initiated disease 20 days post inoculation. Symptoms on the resistant genotype started as mild mosaic and leaf chlorosis. These symptoms were developed on few leaves of the inoculated plants. There was no increase in the disease severity till the end of the experiment (60 days post inoculation). Similar observations of delayed symptom expression followed by symptom suppression in resistant cultivars were made by other workers in different plant-virus systems [2 and 15].

3.2. Phenol content

Pusa Nanha on inoculation exhibited a significant increase in phenol content over the un inoculated plants with the maximum value of 10.21 mg g^{-1} recorded at 10 DAI. However, protein contents were on par in both inoculated and uninoculated plants of local variety, with maximum value of 5.51 mg g^{-1} recorded at 60 DAI. Except at 5 DAI, at all the other stages of inoculation, the amount of total phenols was significantly higher in leaves of inoculated plants of Pusa Nanha, compared to the uninoculated plants and plants of susceptible genotype (Figure 1).

The high level of phenolic content in these plants can be correlated with increased resistance to PRSV, as the accumulation of total phenols was reported to be higher in resistant genotypes compared to susceptible ones [8, 14 and 16].



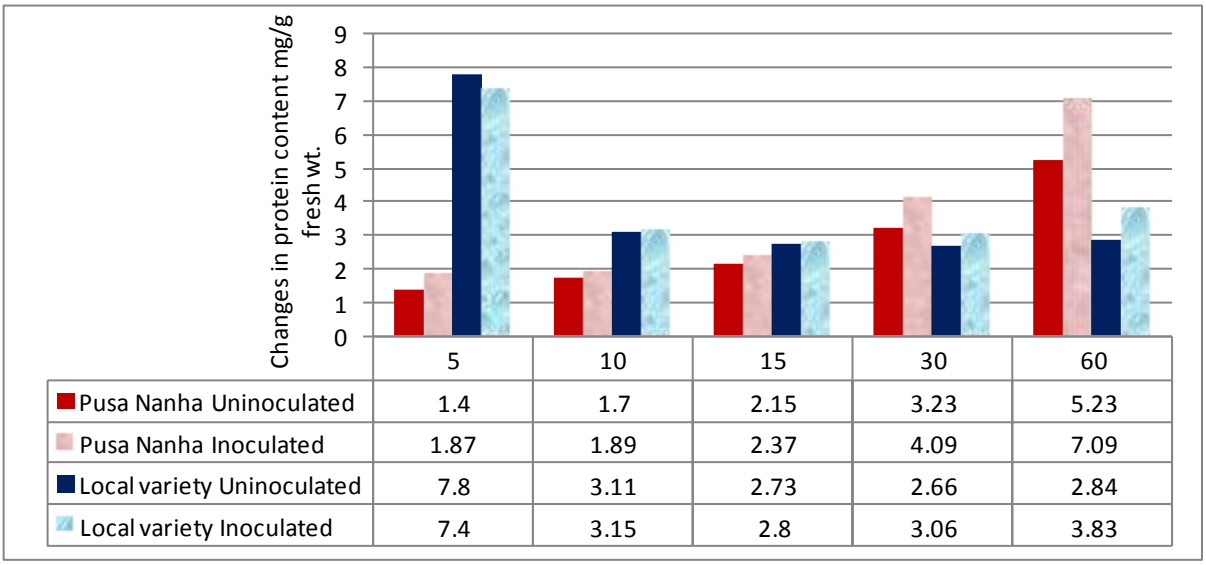
	5	10	15	30
Pusa Nanha Uninoculated	2.4	9.39	4.61	2.43
Pusa Nanha Inoculated	3.21	10.21	6.97	3.03

Figure 1.Changes in phenol content of Pusa Nanha and local variety leaves in response to virus inoculation

3.3. Total protein content

The initial protein content of 1.87 mg g^{-1} which was observed in Pusa Nanha at 5 DAI, increased to a maximum of 7.09 mg g^{-1} at 60 DAI. In case of susceptible variety, protein content lowered from 7.40 mg g^{-1} at 5 DAI to 3.83 mg g^{-1} at 60 DAI. A significant increase was noticed for the inoculated plants of Pusa Nanha, compared to the uninoculated plants. Local variety showed no significant difference in protein content at 10 and 15 DAI, compared to uninoculated plants (Figure 2).

Concurrent with the findings of present study, there are previous reports on PRSV infection resulting in increased total protein content in papaya leaves [7, 17, 18 and 20]. Usually, infected plants show a high protein content, which could be due to activation of both host defense mechanisms and the pathogen attack mechanisms (Agrios, 1997).



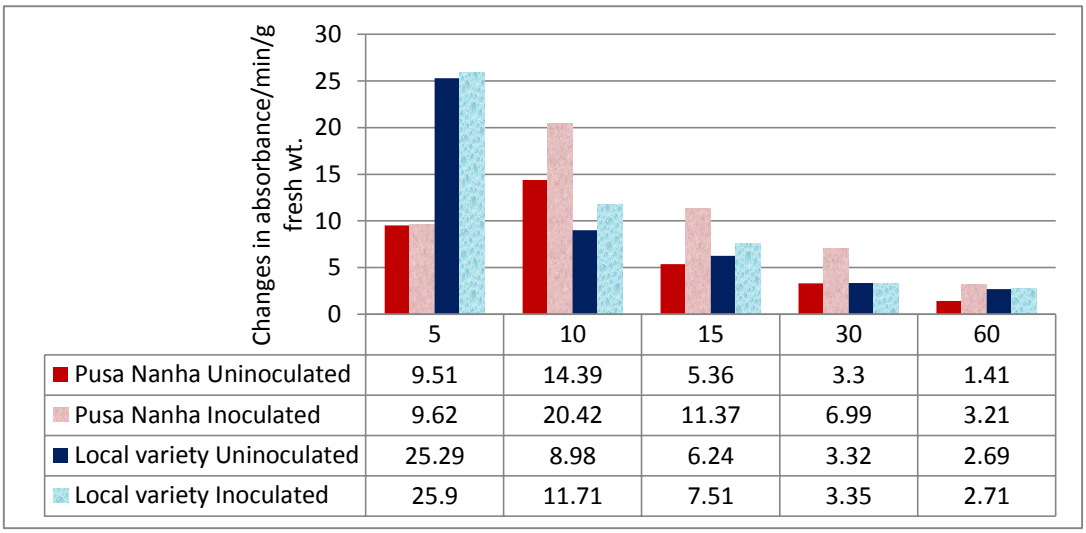
	5	10	15	30	60
Pusa Nanha Uninoculated	1.4	1.7	2.15	3.23	5.23
Pusa Nanha Inoculated	1.87	1.89	2.37	4.09	7.09
Local variety Uninoculated	7.8	3.11	2.73	2.66	2.84
Local variety Inoculated	7.4	3.15	2.8	3.06	3.83

Figure 2.Changes in total soluble protein content of Pusa Nanha and local variety leaves in response to virus inoculation

3.4. Peroxidase activity (PO)

Pusa Nanha exhibited the maximum PO activity of 20.42 $min^{-1}g^{-1}$ at 10 DAI, which lowered to 3.21 $min^{-1}g^{-1}$ at 60 DAI. Susceptible variety on inoculation resulted in a significant higher activity of 25.90 $min^{-1}g^{-1}$ at 5 DAI, which sharply declined to 2.71 $min^{-1}g^{1}$ at 60 DAI. Except at 5 DAI, at all the other days of inoculation, PO activity was significantly higher in inoculated plants of Pusa Nanha, as compared to uninoculated plants and the susceptible genotype. The activity was on par with the un inoculated plants, 5 days post infection. Inoculated plants of susceptible genotype showed an initial significant increase in enzyme activity which was lost at 30 DAI and 60 DAI (Figure 3).

Mydlarz and Harvell [13] have also reported a greater increase in peroxidase activity in resistant plants compared to the susceptible ones.



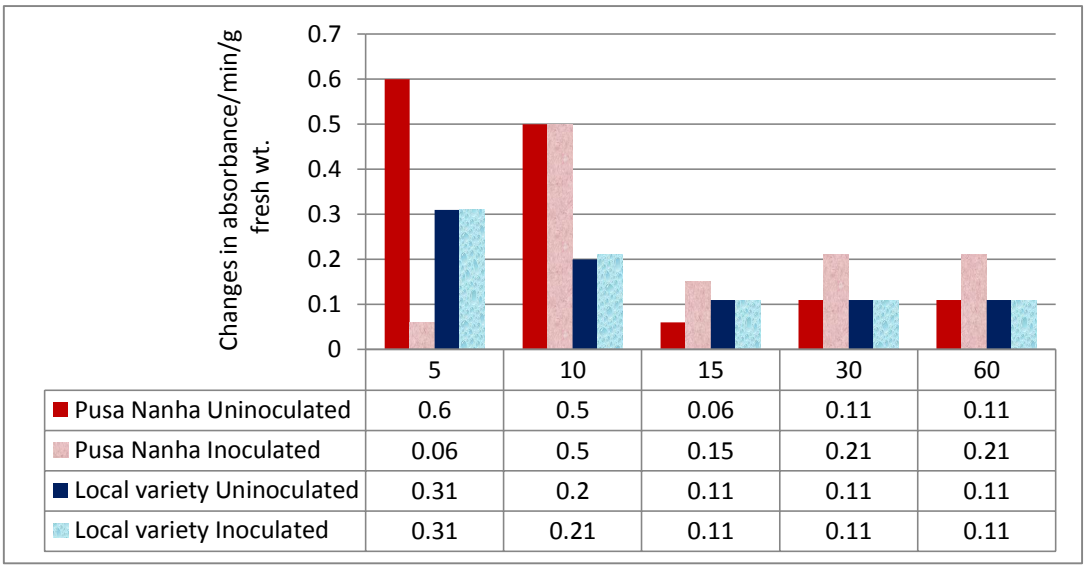
	5	10	15	30	60
Pusa Nanha Uninoculated	9.51	14.39	5.36	3.3	1.41
Pusa Nanha Inoculated	9.62	20.42	11.37	6.99	3.21
Local variety Uninoculated	25.29	8.98	6.24	3.32	2.69
Local variety Inoculated	25.9	11.71	7.51	3.35	2.71

Figure 3.Changes in peroxidase activity of Pusa Nanha and local variety leaves in response to virus inoculation

3.5. Polyphenol oxidase activity (PPO)

The PPO activity was found to increase significantly for the inoculated plants of Pusa Nanha, except at 10 DAI, compared to the susceptible genotype. The maximum activity of 0.50 $min^{-1}g^{-1}$ was noticed at 10 DAI which lowered to a stable value of 0.21 $min^{-1}g^{-1}$ at 30 DAI and 60 DAI.The enzyme activity showed a nonsignificant difference for the inoculated plants of susceptible variety, compared to the uninoculated counterparts. Here the maximum activity of 0.31 $min^{-1}g^{-1}$ was noted at 5 DAI which decreased to a minimum value of 0.11 $min^{-1}g^{-1}$ at 15 DAI, 30 DAI and 60 DAI. Enzyme activity was recorded the maximum for inoculated plants of Pusa Nanha, except at 5 DAI, were the maximum activity was noted for the uninoculated resistant plant (Figure 4).

Chang and Yan [5] have also, reported that, PRSV infection resulted in higher activities of peroxidase and polyphenol oxidase, in the natural resistant mutant of PRSV as compared to susceptible cultivar, Suizhonghong.



	5	10	15	30	60
Pusa Nanha Uninoculated	0.6	0.5	0.06	0.11	0.11
Pusa Nanha Inoculated	0.06	0.5	0.15	0.21	0.21
Local variety Uninoculated	0.31	0.2	0.11	0.11	0.11
Local variety Inoculated	0.31	0.21	0.11	0.11	0.11

Figure 4.Changes in polyphenol oxidase activity of Pusa Nanha and local variety leaves in response to virus inoculation

3.6. Phenylalanine ammonia-lyase activity (PAL)

PAL activity of C06 reached the maximum of 105.90 µg $g^{-1}min^{-1}$ at 15 DAI, which lowered to 75.44 µg $g^{-1}min^{-1}$ at 60 DAI. Inoculated plants of the susceptible local variety showed the highest activity of 76.64 µg $g^{-1}min^{-1}$ at 5 DAI, which gradually declined to 49.20 µg $g^{-1}min^{-1}$ at 60 DAI. The results revealed that, enzyme activity was significantly higher in the inoculated plants of Pusa Nanha, compared to the uninoculated plants and the inoculated plants of susceptible genotype. On the other hand the activity was found to decrease significantly in the inoculated plants of susceptible genotypes, as compared to the uninoculated control plants (Figure 5).

Similar increase in PAL activity in resistant cultivar, was observed in cotton infected with *Cotton leaf curl virus* [15]. A possible explanation for decrease in enzyme activity after PRSV inoculation in susceptible genotype is, many plant pathogens could actively suppress the expression of plant defense reactions during successful infection [11].

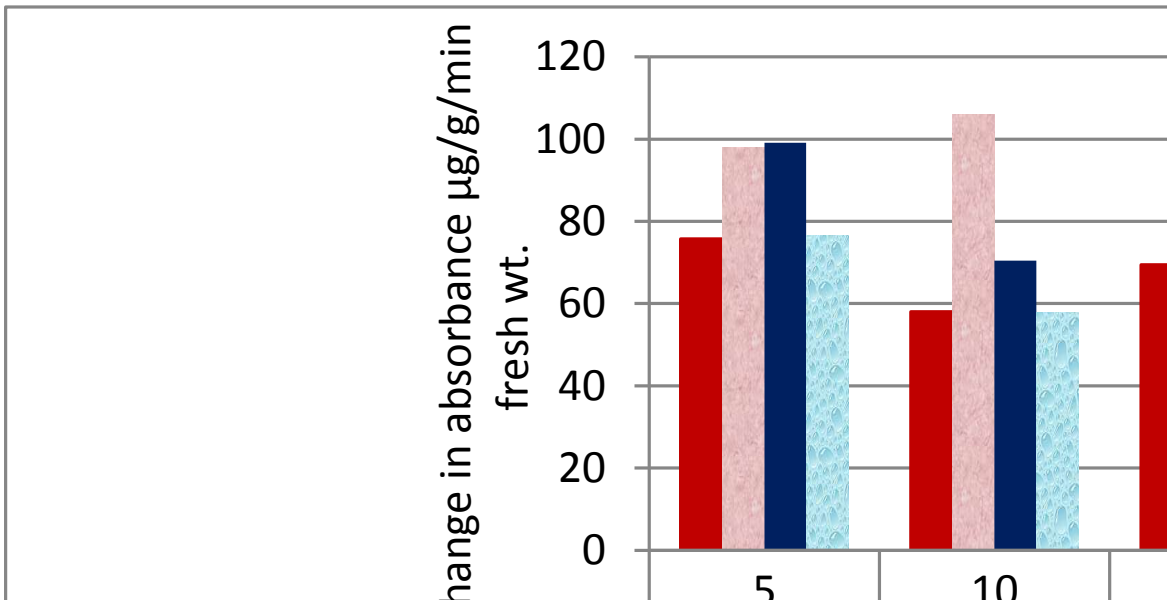


Figure 5.Changes in phenyl alanine ammonia-lyase activity of Pusa Nanha and local variety leaves in response to virus inoculation

IV. CONCLUSION

Our present findings indicated that phenol, total soluble protein, peroxidase, polyphenol oxidase and phenyl alanine ammonia-lyase have an active role in disease resistance to PRSV, owing to their significant increase in inoculated plants of resistant genotype. This study points for the possible use of these components as biochemical markers for investigating compatible and incompatible plant-virus interactions.

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