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STUDIES ON HISTOCHEMICAL CHANGES IN PAPAYA (*CARICA PAPAYA* L.) INFECTED BY PAPAYA RING SPOT VIRUS (PRSV).

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ABSTRACT

Papaya Ring Spot Virus is a RNA containing virus transmitted mechanically and by numerous species of Aphids in a non-persistent manner. Multiplication of virus particles in the infected plant cells alters biochemical compounds of cells such as chlorophyll, β-carotene, organic carbon, nucleic acids etc .External manifestations of disease symptoms are the results of altered host metabolism. The extent of crop loss is mainly associated with severity of visible symptoms. The diseased plant having moderately rich polysaccharides in leaves in comparison to rich polysaccharides in healthy leaves. The protein content of infected papaya plants show high significant increase in comparison to healthy plants in the total content of protein. The amount of different free amino acid also decreases in infected papaya plants significantly than that of the healthy ones . Greater understanding of these biochemical changes may increase the accuracy of disease loss assessment, which helps to know about the nature of the virus.

Keywords-Papaya Ring Spot Virus, protein, amino acids.

1. Introduction

Papaya (*Carica papaya* L.) is a tropical and subtropical economically important food crop widely cultivated throughout the country for fruits belonging to the small family Caricaceae. Papaya Ring Spot Virus is a most devastating and wide spread throughout the India. The incidence of ring spot disease has been reported from several parts of India, causing a fruit

loss upto 70%. The incidence of the disease has also been reported up to 95% from eastern U.P. (Khurana 1968, 1974).

Papaya ring spot virus (PRSV) is the most wide spread and devastating on papaya throughout India. In the past, PRSV was severe in Gujarat, Bihar, Maharashtra Uttar Pradesh and Goa, but no its incidence is high and spreading rapidly to southern states Andhra Pradesh and Karnatka (Muniyappa, 2003). Its incidence ranges from 40 to 100% depending on the season and weather. It has threatened commercial cultivation and papaya based industries in India.The reduction of polysaccharide may be due to varied metabolism in infected tissue. The papaya is regarded as a fair source of iron and calcium; a good source of vitamins A, B and C and an excellent source of vitamin C (ascorbic acid). Several workers have reported significant decrease in total sugars, reducing sugars & non-reducing sugars in diseased papaya leaves (Singh, 1973).

The present study was undertaken to determine changes in concentration of protein and free amino acids in Papaya infected by Papaya ring spot virus (PRSV) under natural condition. The change in amino acid and protein content reduces the quality of fruit for which it is well known throughout the world.

2. **Lliterature**

Papaya Ring Spot Virus (PRSV) is a type of potyvirus that infects papaya and a few closely related species. Potyviruses are the most economically damaging plant viruses. Aphids spread PRSV by feeding off infected and then uninfected plants. The virus is not passed on by the fruit - the offspring of an infected plant are usually virus free until they are inoculated with the virus by aphids carrying PRSV. There are many variants of PRSV that are closely related but have differences in genetic sequence and virulence. Papaya Ring Spot Virus is a virus with RNA-containing, flexuous filamentous particles *c.* 780 x 12 nm.

The incidence of papaya ring spot virus (PRSV) disease has become a major limiting factor affecting economy of farmers. The disease initially appearing as mosaic on leaves totally distorts leaves and entire plants in advanced stage causing a fruit loss upto 70% .A dramatic biochemical changes in virus infected plants result in decrease of both quality and quantity of infected crops. Various reports suggest that virus multiplication inside the plant cell alters different biochemical constituents of plants and disrupt the physiological process like

photosynthesis, transpiration and respiration of the infected plants which affect the growth and yield (Tajul, 2011).

Plant viruses cause the degradation of chlorophyll content in virus infected plants (Bertamini *et al.*, 2003 and Hemida, 2005). The sugars play pivotal roles in the life cycle of plants.The sugar induced feedback inhibition of photosynthesis is one example of a sugar regulated process. When carbohydrates accumulate in mature source leaves, repression of genes involved in photosynthesis is observed and as a consequence photosynthesis is reduced. Kunkalikar *et al.*, (2007) find that, Papaya ring spot virus brings about histological and histochemical changes in papaya upon infection. In diseased leaves, palisade cells were markedly distorted. The spongy cells lost their normal round shape with complete disintegration

The histopathological & histochemical changes associated with various morphological symptoms produced by PRSV has not been described in details even though they are important to understand the disease development and host pathogen relationship.

3. **Methodology**

The papaya seedlings will be raised from the seed of different varieties of papaya taken from local seed growers and NBPGR New, Delhi. Seedlings were raised in earthen pods of 6 inch diameter filled with mixture of soil and farm yard manure (1:1 w/w). The soil mixture will be sterilized before raising the plant in it. All the experiment will be conducted in an insect proof chamber under normal day light. The plants were grown in a greenhouse in the campus of Botany Department, M. L. K.P.G.College, Balrampur.

Virus inoculum was the crude sap obtained by trituration of frozen leaves of papaya plants showing mosaic symptoms as maintained in the Botany Department green house as standard inoculum. Inoculation of leaves was carried out by rubbing with finger after their being dusted with carborandum as described by Rawlinsand Tompkins, (1936). The two month old plant will be sap inoculated the plant showing typical diseased symptoms will be selected after 25 days of inoculation.

For histochemical studies Healthy and infected plants were collected in the morning, each sample was analyzed twice. Protein content was determined according to micro Kjeldahles as outlined by A.O.A.C. (1995). Free amino acids content mg/g dry weight was determined calorimetrically according to the method described by Jayarman (1981). The individual amino

acids present in the healthy and infected leaves were identified by Chromatography according to Moore *et al.*, (1958).

4. Results/Findings

The proteins are complex polymer of amino acid with high molecular weight. They are most important chemical constituents of living organisms. Data in Table (1) shows that, (PRSV) infected papaya plants show high significant increase of protein in comparison to healthy plants in the content of total protein. There was a progressive increase in protein contents of healthy and infected plants with increase in plant age. PRSV inoculated plants recorded 7.93, 8.53, and 19.27 percent increase in protein content over healthy plants at first, second and third week's respectively after inoculation.

Table 1

Weeks after inoculation	Protein content(mg/g)	Protein content(mg/g)	
	Healthy	Infected	Percent increase over healthy
First week	0.62	0.67	7.93
Second week	0.84	0.90	8.53
Third week	0.84	1.00	19.27

Effect of PRSV infection on protein content of papaya plants at different weeks after inoculation.

Data in Table (2) shows that, (PRSV) infected papaya plants contain significant lower content of total free amino acids than that of the healthy ones. PRSV inoculated plants recorded 32.61, 57.01, and 24.18 percent decrease in free amino acids content over healthy plants at first, second and third week's respectively after inoculation.

Table 2..

Weeks after inoculation	Amino acids content(mg/g)		
	Healthy	Infected	Percent decrease over healthy
First week	3.69	2.78	32.61
Second week	9.58	6.10	57.01
Third week	8.42	6.78	24.18

Effect of PRSV infection on amino acids content of papaya plants at different weeks after inoculation.

(Data in the Table 1, 2 are statistically verified)

5. Discussion

The total free amino acid increases with age in the healthy and diseased plants but percentage increase is more in diseased plants. The table-2 shows that after one, two and three week its percentage increases by 32.61, 57.01 and 24. 18. This result was agreed with that obtained by Patel et.al (2004).

Protein contents were higher in diseased leaf as compared to healthy leaf. Similar results were observed by Prakash *et al*. (1995) in leaves and seeds of *Amaranthus* and *Chenopodium* infected with cucumber mosaic virus and Meena et,al.(2016)in *Capsicum annum.* Cheema *etal*., (2003) showed that, protein content in two soybean varieties increased with infection with soybean yellow mosaic virus. Haque et.al(2005)reported that changes in chemical composition and respiration of Zuchini Yellow Mosaic Virus infected pumpkin leaves revealed that ZYMV infection increased the protein content of pumpkin leaves compared to healthy ones. Muqit *et al*., (2007) showed that, in ash gourd (*Benincasa hispida*) due to infection of *Papaya ring spot virus* (PRSV) total protein was increased in the infected leaves in comparision to healthy ones. Singh *et al*., (2007) reported that, primary and secondary metabolites like proteins have received considerable attention in relation to resistance in plants against diseases. Protein content was higher in plant parts infected with *Pea mosaic virus* (leaf, stem and root) than their healthy

counterparts, but maximum protein content was found in diseased leaves followed by root and stem. Poonam and Gupta (2008) reported that, the infected samples of bean plant infected with bean common mosaic virus contained more protein than healthy ones. Rao *et al*., (1989) concluded that, the increased protein content in virus infected plants was due to increased activity of RNA synthetase or RNA polymerase.

The decrease in amount of amino acid in disease plants was agreed with that obtained by Jeyarajan and Ramakrishnan (1972); Chowdhury *et al.*, (1985). Fiebig *et al.* (2004) found a significant reduction in the concentration of the total amount of amino acids of barley plants infected with Barley yellow dwarf virus. The decrease in total free amino acids may be due to the reduction in photosynthesis and the increase in respiration rate. Hemida (2005) showed that, virus causing mosaic, mottling, malformation and distortion in faba bean (*V. faba*) was found in various fields in Assiut Governorate, Egypt. The virus isolate was detected and identified as *Bean yellow mosaic virus* (BYMV). Total free amino acids were estimated in leaves of two host plants (*Vicia faba* and *Phaseolus vulgaris*) inoculated with BYMV. In *Vicia faba* and *Phaseolus vulgaris* plants, the virus isolate induced a lower concentration in free amino acids.

6. Implication to Research and Practice

The incidence of papaya ring spot virus (PRSV) disease has become a major limiting factor affecting economy of farmers. The disease initially appearing as mosaic on leaves totally distorts leaves and entire plants in advanced stage causing a fruit loss upto 70% .The incidence of disease has also been reported up to 95 % from eastern U.P. (Khurana *et al.* 1968, 1974).

Multiplication of virus particles in the infected plant cells alters biochemical compounds of cells such as chlorophyll, β-carotene, organic carbon, nucleic acids etc. External manifestations of disease symptoms are the results of altered host metabolism. The extent of crop loss is mainly associated with severity of disease. Greater understanding of these biochemical changes may increase the accuracy of disease loss assessment, which helps to know about the nature of the virus and their management.

7. Conclusion

Viruses are obligate intracellular parasite use the host enzymatic machinery for their own metabolic processes. The increase in total protein content and decrease in free amino acids proves that virus induces the synthesis of new molecules which was not required by host that is

addition of new biomolecules in protoplasm that leads to change in chemical constituent of host cell .Thus virus infection changes the quality as well as quantity of yield that is economic loss to individual as well as society. Thus study of virus is not only for virology & pathology but more important to understand basic principle of plant virus interactions for future of disease management.

8. Future Research

Papaya constitutes one of the most important fruit of the society throughout the country. Viruses are obligate intracellular parasite parasitizing plants as well as animals. The viruses are characterized by their morphological, physiological, & genetical characters. Most of the viral diseases are identified by morphological deviations in host plant but anatomical and histochemical deviations helps further in discovery of new pathogens that will be helpful in disease management as well as variety improvement to fulfill the growing demand of organics, protein and other therapeutics environfriendly.

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