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Effect of *Papaya Ring Spot Virus* (PRSV) infection on nitrogen, protein and carbohydrate contents in papaya

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ABSTRACT

The present investigation was undertaken to assess the impact of PRSV infection on assimilation of nitrogen, protein and carbohydrates in the vegetative parts of the host *Carica papaya* L. The nitrogen and protein contents were higher in infected tissue compared to healthy counterparts. The nitrogen and protein content was maximum in leaves and minimum in roots (for both healthy and diseased samples). Initially, there was a very slight increase in nitrogen and protein content values due to infection, but a progressive rise was noted in older tissues. However, the carbohydrate content including reducing sugars, non-reducing sugars and starch, was found to be lower in infected tissues compared to their healthy counterparts, but the carbohydrate content value of old tissues was higher than the younger ones (for both healthy and diseased samples). The data was statistically significant and results were critically discussed. It is concluded that PRSV infection stimulates biosynthetic pathways for increased synthesis of amino acids for viral requirements resulting in rise in nitrogen and protein content causing biosynthesis of amino acid due to breakdown of assimilated carbohydrates, which are reduced due to infection. Reduced productivity in leaves and poor assimilation of carbohydrates results in heavy yield loss.

Key words: PRSV, nitrogen, protein, carbohydrates, papaya.

INTRODUCTION

Papaya Ring Spot Virus (PRSV), a member of genus *Potyvirus* (Brunt *et al*., 2) causes one of the most destructive diseases, the papaya ringspot, which has restricted the cultivation of papaya (*Carica papaya* L.) in the tropical and sub-tropical countries. The incidence of PRSV disease has been reported from several parts of India, causing yield loss up to 70 to 95% (Khurana, 5; Singh, 9). The virus induces symptoms of chlorotic mottling, ringspot and distortion on foliage, stem and fruits and also on the roots of host. The symptoms are severe in winters (at 15°-20°C), and very mild in summers (at 35° - 40°C). The particle morphology studied by electron microscopy reveals flexuous rod-like particles (760 × 12 nm), tested positive with antiserum of PRSV in DAC-ELISA and DIBA tests. The virus isolate has a Dilution end point (DEP) 10^{-4}, Thermal inactivation point (TIP) 65°C and Longevity *in vitro* (LIV), less than 2 days, with a narrow host range on Caricaceae and Cucurbitaceae. The virus is stylet borne and is naturally transmitted in a non-persistent manner by aphids, *Aphis craccivora, A. gossypii, A. citricola, Myzus persicae* and *Rhopalosiphum maydis*, most common in the study region *viz*., eastern Uttar Pradesh (Singh, 9).

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In view of the heavy losses caused due to the PRSV isolate in the eastern U.P., the present investigation was undertaken to study the effect of virus infection on nitrogen, protein and carbohydrate contents in the leaves, stem and roots of the host papaya.

MATERIALS AND METHODS

To study the effect of PRSV infection on the nitrogen, protein and carbohydrate content in vegetative parts of the papaya cv. Honey Dew, an experiment was conducted in 2008 under glasshouse conditions. Papaya seedlings were raised in ten small pots (8") with five seedlings each. Five of the pots were inoculated with the Standard Inoculum (SI), following routine procedure. A healthy control was also maintained. Plants were maintained with proper sanitation and recommended farm yard manure and fertilizers. All inoculated plants were tested in DAC-ELISA after 15 days and 90 days after inoculation, with polyclonal antiserum of PRSV.

For estimation of nitrogen, protein and carbohydrate contents, leaf, stem and root samples were harvested from the test plants at an interval of 15 days. The first harvesting was done 15 days after inoculation (at the appearance of symptoms) followed by 30, 45, 60, 75 and 90 days (with no apparent increase in symptoms). To estimate total nitrogen content, 0.1 g of oven-dried leaf tissue was extracted 2-3 times with 80% ethanol. The aqueous ethanol fraction containing soluble

nitrogen and residues forming insoluble nitrogen fraction were separated by centrifugation at 3,000 rpm and collected separately. The solvent was evaporated at 80°C and dried material was digested with 1 ml of digestion mixture (1 g salicylic acid/20 ml of conc. H_2SO_4), mixed well and allowed to stand for 20 min. then treated with 4 drops of 50% sodium thiosulphate solution slowly until fumes appeared, thereafter, cooled and treated with 1 ml of $CuSO_4$ (per 500 ml of 60% perchloric acid), heated to boiling till digestion of material was completed. Volume was made up to 50 ml by adding distilled water. To 1 ml of the above solution, distilled water and Nesseler's reagent (1:1) were added and OD read at 420 nm by Carl Zeiss Jena Specol Model 10 colorimeter. Protein content was estimated by method described by Snell and Snell (10). Approx. 0.1 g oven-dried leaf tissue was crushed with 10 ml of trichloroacetic acid and centrifuged at 1,600 rpm for 30 min. and filtered. The residue was digested by Doneen's method and colour was developed as above. The value obtained was multiplied by a factor 6.25 to obtain the total protein content.

To estimate the reducing sugar in the leaves, 0.1 g of oven-dried leaf tissue was extracted in different grades of alcohol 80, 60, 40, 20% and finally distilled water. Initial extraction with 80% alcohol was done for 30 min. in boiling water bath with a reflex condenser (overnight at room temperature). While extracting with lower grades of alcohol, 2 drops of toluene were added to prevent microbial activity. After last extraction with distilled water the solution was filtered and filtrate clarified, evaporated to a volume of 1 ml and treated with 1 ml basic lead acetate. The precipitate was filtered and treated with 3 ml of saturated potassium oxalate to precipitate out excess of lead, again filtered and volume made up to 5 ml by distilled water. Reducing and non-reducing sugars were estimated (Somogyi, 11).

Starch content was estimated by method of Snell and Snell (10). About 50 mg of dried leaf tissue was homogenized with 10 ml of 40% perchloric acid, homogenate was clarified with charcoal, centrifuged at 6,000 rpm and filtered. To 1 ml of the above filtrate, 1 ml of 5% potassium iodide solution was added. After 5 min., 1 ml of 0.04% potassium iodate was added. The mixture was allowed to react. The colour intensity was read on colorimeter at 620 nm.

RESULTS AND DISCUSSION

Data presented in Tables 1, 2 and 3 reveals that the ethanol soluble nitrogen content is higher in diseased plant, i.e. 1.91, 0.80 and 1.14 mg/100 mg dry wt. for leaves, stem and root tissues, respectively compared to healthy samples, 1.58, 0.71 and 0.95, respectively. Similarly, ethanol insoluble component was higher in diseased tissues 2.66, 1.81 and 1.72 mg/100 mg dry wt. for leaf, stem and root samples, respectively as against 2.39, 1.68 and 1.56 for their healthy counterparts. The data was significant at P = 0.05. This is due to accumulation of insoluble proteins, free amino acids, nucleic acids, amines and inorganic nitrogen. There is increased demand for glycine and serine during the synthesis of capsids and nucleic acids, as earlier also confirmed by Sarkar *et al.* (6), and Shukla (7). The biosynthetic pathways are thus stimulated for enhanced synthesis of limiting amino acids, rather than those already present in excess of viral requirements, which are then synthesized and accumulated in infected tissues resulting in increased nitrogen content. Further, we found that initially there was very slight increase in nitrogen content in infected tissues because of maximum viral synthesis. Later, with time and in older infected tissues, this concentration greatly increases because viral synthesis slows down and there is accumulation of amino acids in infected tissues leading to a progressive rise in nitrogen content with age. Nitrogen content was maximum in leaves [average value 2.06% (ethanol soluble) and 1.70% (ethanol insoluble)] and minimum in roots [average rise 1.16% (ethanol soluble) and 0.99% (ethanol insoluble)]. This is because translocation of nitrogen from leaves to other parts occurred in the form of amides (glutamine) and this transfer is retarded due to damage of translocation facility (xylem and phloem) in infected tissues which results in accumulation of glutamine in leaves higher than in roots. Further more, this results in depletion of nitrogen from growing points causing stunting and chlorosis symptoms.

The results of the findings reveal that due to the PRSV infection, Hill reaction is impaired, causing a reduced productivity in chlorotic tissues. The chlorophyll protein is utilized for viral protein synthesis and there is increase in cytoplasmic protein content in diseased tissue 16.47, 11.18 and 10.49 mg/100 mg dry wt. for leaf, stem and root samples compared to the healthy counterparts 14.84, 10.41 and 9.54 mg/100 mg dry wt., respectively. Data were statistically significant. These findings are in confirmation to those of Sun (13), and Singh *et al.* (8). The increased protein synthesis is due to increased production of enzyme RNA synthetase (RNA polymerase) which stimulates the production of viral protein. The protein content increases with age due to enhanced requirement of enzymes and other proteinaceous substance for senescence.

However, it was observed that carbohydrate content was found to be reduced in infected tissue (13.60, 8.13 and 5.25 mg/100 mg dry wt.) for reducing sugars in leaf, stem, and root samples, respectively and for non-reducing sugars (8.43, 5.88 and 2.63%) for leaf,

Table 1. Effect of PRSV infection on nitrogen, protein and carbohydrate contents (mg/100 mg dw) in papaya leaves.

DAI	Nitrogen content						Protein			Carbohydrates								
	Ethanol-soluble			Ethanol-insoluble						Reducing sugars			Non-reducing sugars			Starch		
	H	D	% increase	H	D	% increase	H	D	% increase	H	D	% reduction	H	D	% reduction	H	D	% reduction
15	0.98	1.06	0.50	2.17	2.26	0.56	13.56	13.93	2.31	9.97	9.52	4.54	7.91	7.83	0.72	14.63	14.19	3.99
30	1.22	1.39	1.06	2.21	2.29	0.50	13.79	14.16	2.31	15.12	13.89	8.11	7.96	7.87	0.81	16.69	15.81	5.72
45	1.56	1.93	2.31	2.33	2.54	1.31	14.31	15.82	9.43	16.31	13.51	13.72	9.02	7.96	10.35	17.13	15.51	7.14
60	1.67	2.23	3.50	2.45	2.81	2.25	15.03	17.31	14.25	17.57	14.61	13.91	10.31	8.46	16.65	18.14	15.59	10.78
75	1.89	2.33	3.75	2.53	2.93	2.50	15.81	18.07	14.12	18.05	14.98	13.81	11.79	9.27	25.20	18.42	15.69	11.05
90	2.21	2.57	2.25	2.67	3.17	3.12	16.57	19.56	18.68	18.43	15.10	14.65	11.97	9.33	26.40	18.56	15.72	11.24
Average	1.58	1.91	2.06	2.39	2.66	1.70	14.84	16.47	10.48	15.90	13.60	11.45	9.82	8.43	9.39	17.26	15.41	8.23
$CD_{0.05}$	0.01	0.05		0.12	0.18		3.05	4.16		3.12	2.99		2.51	2.00		3.11	3.70	

DAI = Days after inoculation.

Table 2. Effect of PRSV infection on nitrogen, protein and carbohydrate contents (mg/100 mg dw) in papaya stem.

DAI	Nitrogen content						Protein			Carbohydrates								
	Ethanol-soluble			Ethanol-insoluble						Reducing sugars			Non-reducing sugars			Starch		
	H	D	% increase	H	D	% increase	H	D	% increase	H	D	% reduction	H	D	% reduction	H	D	% reduction
15	0.45	0.52	0.37	1.28	1.37	0.5	7.92	8.46	3.37	6.61	6.52	2.92	4.53	4.43	0.72	11.07	10.73	2.90
30	0.57	0.67	0.5	1.41	1.48	0.5	8.73	9.22	2.87	7.76	7.12	4.29	5.55	4.93	5.76	11.16	10.78	2.25
45	0.69	0.72	0.18	1.53	1.67	0.87	9.43	10.11	4.25	8.81	7.44	6.81	5.73	5.08	5.67	12.17	10.83	5.90
60	0.82	0.83	0.19	1.80	1.96	1.00	11.10	12.03	5.62	10.97	9.03	9.21	7.84	6.67	10.53	13.23	11.77	6.17
75	0.87	0.92	0.31	1.97	2.09	0.75	12.18	13.09	5.62	11.32	9.25	9.49	8.12	6.93	13.32	13.49	11.83	8.01
90	0.89	0.95	0.37	2.10	2.31	1.31	13.07	14.17	6.87	11.93	9.63	10.34	8.74	7.23	13.86	13.93	11.92	8.55
Average	0.71	0.80	0.31	1.68	1.81	0.82	10.41	11.18	4.76	9.57	8.13	7.17	6.75	5.88	8.31	12.51	11.31	5.63
$CD_{0.05}$	0.01	0.015		0.01	0.05		2.20	2.33		2.20	1.90		1.51	1.33		2.66	2.50	

DAI = Days after inoculation.

Table 3. Effect of PRSV infection on nitrogen, protein and carbohydrate contents (mg/100 mg dw) in papaya root.

DAI	Nitrogen content						Protein			Carbohydrates								
	Ethanol-soluble			Ethanol-insoluble						Reducing sugars			Non-reducing sugars			Starch		
	H	D	% increase	H	D	% increase	H	D	% increase	H	D	% reduction	H	D	% reduction	H	D	% reduction
15	0.65	0.71	0.37	1.43	1.51	0.5	8.85	9.28	2.67	4.43	4.23	1.91	2.33	2.11	1.8	6.32	5.97	3.18
30	0.73	0.86	0.81	1.49	1.63	0.87	9.17	9.92	4.68	5.37	4.95	2.70	2.38	2.21	1.44	7.43	6.22	7.06
45	0.87	1.11	1.37	1.54	1.68	1.00	9.43	10.28	5.56	5.43	5.03	2.05	3.39	2.61	6.84	7.49	6.26	5.20
60	1.13	1.26	0.81	1.59	1.76	1.00	9.63	10.75	7.00	6.37	5.43	4.46	3.47	2.65	7.38	8.53	6.65	7.78
75	1.15	1.38	1.5	1.63	1.83	1.37	9.94	11.18	7.81	6.83	5.75	4.68	4.63	3.09	15.50	8.64	6.71	7.79
90	1.17	1.51	2.12	1.70	1.89	1.25	10.23	11.54	8.18	7.22	6.13	4.88	4.63	3.13	15.60	9.65	7.63	7.92
Average	0.95	1.14	1.16	1.56	1.72	0.99	9.54	10.49	5.98	5.94	5.25	3.44	3.48	2.63	8.09	8.01	6.57	6.49
$CD_{0.05}$	0.01	0.02		0.05	0.07		1.20	1.51		0.89	0.80		0.66	0.45		1.12	1.06	

DAI = Days after inoculation.

stem and root samples, respectively and 15.41, 11.31 and 6.57% for starch content, respectively compared to (15.90, 9.57 and 5.94%) (reducing sugars), (9.82, 6.75 and 3.48%) (non-reducing sugars) and (17.26, 12.51 and 8.01%) (starch) in their healthy counterparts. The data was statistically significant. The reduced chlorophyll synthesis influences synthesis of hexoses (sucrose and starch). During carbohydrate metabolism, there is synthesis of amino acids and proteins from intermediary products. As confirmed by our findings, nitrogen content increased due to PRSV infection. This is due to increased demand for viral protein synthesis which results in diversion of assimilated carbon. The synthetic phase of photosynthesis is anabolic counterpart of respiration. Thus, an increased breakdown of carbohydrates occurs in the diseased tissues compared to healthy ones, thus, reducing the rate of synthesis of starch and enhancing breakdown of sugars which are converted to phosphate esters and serve as substrates for enhanced rate of respiration (Singh, 9). The different levels of carbohydrates reducing and non-reducing sugars in the different parts of the infected host was found to be 11.45 and 9.39% (leaves), 7.17 and 8.31% (stem) and 3.44 and 8.03% (roots) respectively. Similarly, the average reduction of starch content was 8.23% (leaves), 5.63% (stem) and 6.49% (roots) respectively in different vegetative parts of the infected hosts. This may be due to disturbed photosynthesis and enzyme transport system. The increase in carbohydrate concentration with age of the plant is due to the fact that increased biosynthesis requires an increased utilization of carbon skeleton, which is often associated with increased activity of necessary enzymes, also supported by Sarkar *et al.* (6).

We conclude that the chlorophyll protein is utilized for viral protein synthesis resulting in depletion of chlorophyll content and increased in cytoplasmic protein. The decreased photosynthetic activity coupled with increased respiratory rate observed here leads to decreased carbohydrate content. The former decreases the biosyntheses and the latter increases the break down of carbohydrates. The biosynthetic pathways are stimulated for synthesis of greater amounts of limiting amino acids, other than those already present in excess of viral requirements which are then synthesized and accumulated in infected areas, leading to a rise in nitrogen content (Tables 1, 2 & 3).

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