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Alteration of Cellular Nutritional Elements and Nucleic Acids of Papaya Leaves Infected with Seven Symptomatic Isolates of PRSV-P

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Abstract: The diverse nature of shift in the amount of cellular nutritional elements and nucleic acids as affected by the infection of different isolates of Papaya Ring Spot Virus-Papaya strain (PRSV-P) was studied in this experimentation. The symptomatic isolates used in the present study were mild mosaic, mosaic, fern leaf, severe mosaic, vein clearing, leaf distortion and chlorotic leaf spot. Parameters measured to quantify the alteration of cellular components comprised different nutritional cellular elements (C, N, P and K) and nucleic acids (DNA and RNA). In all occasion of measured parameter, the highest alteration was found in leaf distortion. Almost in all cases the lowest effect was determined with the Mild Mosaic infected plants followed by the mosaic, severe mosaic, vein clearing, chlorotic leaf spot, fern leaf and leaf distortion. Infection of seven different symptomatic isolates of PRSV-P created striking and responsive variability in all parameters studied. And the dependency of the cellular nutritional elements and nucleic acid on seven symptomatic variants of PRSV-P was found significantly correlated in respect to the corresponding yield.

Key words: PRSV-P, symptomatic isolates, cellular components, nucleic acid

INTRODUCTION

Viral infection occurs physically at subcellular level, functionally for macromolecule synthesis and establish through viral genomic action operating the metabolic activity of the invaded host cell. Consequently, massive and diversified shift happen in various cellular constituents, chiefly in cellular nutritional elements and nucleic acid, which afterwards causes aberration in physiological processes and finally manifesting various precise symptoms and severity of the disease corresponding to the virus genome specific in role (Bos, 1967, 1982; Bawden, 1959; Diener, 1963; Matthews, 1991; Porter, 1959; Sadasivan, 1963). Viral diseased symptoms and severity to a large extent specific to viral pathogenicity which is an intrinsic property of the virus (Singh *et al*., 1994). Generally stunting/growth reduction is related with the retarded production and function of the cellular components (Zaitlin, 1987).

The alteration (either increase or decrease) of constitutional/structural cellular components like organic carbon, nitrogen, protein, phosphorus, potash etc. has been reported to happen due to infection of virus, although the degree of change depends largely on the virus in role and to some extent on the

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host. Such depletion or increase has obviously been related with the type of symptoms developed, severity and nature of damage to the crops (Ayers, 1981; Bawden, 1959; Diener, 1963; Goodman *et al*., 1986; Heitefuss and Williams, 1976; Matthews, 1991; Porter, 1959; Sadasivan, 1963; Wood, 1982; Wynd, 1943). The study on Biochemical changes induced by *Papaya ring spot virus* by Sarker *et al*. (1995) reported that infected leaves contained higher levels of nitrogen and slightly more sugar and free amino acids than healthy leaves, while dry matter, orthodihydric acid and total phenol contents were reduced.

All the events of constituting a new virus particle required the utilization of nucleic acid and protein synthesis mechanism of the infected host cell which results the obvious alteration of DNA and RNA level of the host cell (Misawa *et al*., 1966; Sreenivasulu, 1989; Takahashi and Ishii, 1952). Whatsoever may be the situations occur due to virus infection in respect to the change of cell components, the marked important point is that the phenomenon is dependent on the degree of pathogenicity of the virus progressing the infection. The mutants of a virus if vary, distinctly among each other promoting themselves to the status of separate variant, the efficiency of altering any of the components by the new variant one should be distinctly apart.

Since, PRSV-P infecting papaya in Bangladesh seems to be appeared as cluster of seven different variants symptomatically (Rahman, 2003), they are supposed to be distinguishable through quantifying the significantly varying cellular nutritional elements and nucleic acid due to infection by each of the symptomatic isolates separately. Therefore, the present investigation was carried out to picturing the diverse alteration in the cellular nutritional elements and nucleic acids from the infection of seven different symptomatic isolates of PRSV-P on papaya in Bangladesh. Prior identification of variants in PRSV-P is important for its management through developing transgenic plants.

MATERIALS AND METHODS

Papaya plants infected with *Papaya ring spot virus*- Papaya strain showing seven different types of symptoms named by the seven symptomatic isolates as described in Table 1 were selected and tagged.

Table 1: Seven symptomatic isolates of PRSV-P strain on papaya leaf in Bangladesh used to conduct test an analysis in this experimentation

Identifying name	Description of principle elements
Mild Mosaic (MM)	Initial symptoms appear in the young leaves as diffuse mosaic and faint yellowing, mostly confined around the whorl of the leaf blade. Less number of inconspicuous green spots appears on the fruits at very late stage of fruit development. Plant looks almost healthy without close observation.
Mosaic (MO)	Systematic scattered mosaic developed on the leaves and covers the whole leaf blades. Streaking on petioles and stems develop prominently. Sharp green spot develop on the fruits. Notable effect on growth of the plant is common 0.
Fern leaf (FL)	Fern like malformed leaf blades appear with mottling. Fruit distortion start with young fruits, more severe on fruit set in late stage of plant growth. Stunting of the plant is quite prominent.
Severe Mosaic (SM)	Mosaic develops with large yellow patches all over the leaf at the initial symptom. The vein clearing restricts near the whorl of the leaf. Water soaked lesion develop on the petioles and stems. Water soaked spot on fruits turn into conspicuous ring spots. Fruits are malformed. Canopy size reduces showing tapering towards apex.
Vein Clearing (VC)	Symptoms initiate with clearing of main veins at the whorl of the leaves, which gradually extends all over veins and inter-veins. There are inter-veinal mild yellowing. Distorted small sized fruits develop numerous ring spots.
Leaf Distortion (LD)	Leaf distortion appear in the young growing leaves initially as irregular serration, blistering of the leaves and severe mottling resulting shoe string, curling of the leaf blade and cupping in same cases. Texture of the leaves formed irregularly and patches become leathery. Petioles and stems are severely affected with streaks and spots.
Chlorotic leaf spot (CS)	Irregularly oval to circular chlorotic spots develop scatteredly on the leaves. When chlorotic area dried up, it resembles the symptom of mites' attack.

Leaf samples were collected from the healthy and infected plants from the similar position and similar age showing distinct symptoms to maintain the sample homogeneity. Samples were also collected at three different growth stages of plants (Early = before fruit setting, mid = fruit setting to first ripening and late = first ripening onward) infected with each symptomatic isolates. The healthy and infected plants were frequently checked for PRSV-P infection using mechanical sap inoculation and DAS-ELISA methods as described by Yeh and Gonsalves (1984) and Clark and Adams (1977), respectively. A double beam spectrophotometer (Model No. 1200-20, Hitachi) was used to measure the absorbance value/optical density (OD) at weave length given by the protocol when it was required. Organic carbon was estimated by weight dilution method developed by Tyurin (1980). Total nitrogen was determined by Kjeldahl method using $CuSO_4$ and K_2SO_4 mixture (1:9) as catalyst following the method of Jackson (1958). The total phosphorus content was estimated by Perchloric acid digestion method (Anonymous, 1975). The content of potassium was measured by following perchloric acid digestion method for mineral elements (Yamakawa, 1992). Content of nucleic acids (DNA and RNA) was determined following the method proposed by Spirin (1958). Change in structural cellular nutritional elements (organic C, N, P and K) and nucleic acids (RNA and DNA) both for healthy and PRSV-P infected leaf samples were taken in every quantifying analysis. Each of the cellular elements were analyzed from 10 samples. Variation among the symptomatic isolates was measured using DMRT (Duncan's Multiple Range Test).

RESULTS

Effect on the Contents of Cellular Nutritional Elements

Significantly ($p<0.05$) the lowest content of organic carbon, nitrogen and phosphorus and the highest content of potassium were recorded in healthy papaya leaves. The contents of the first three structural cellular components increased significantly ($p<0.05$) due to infection with all the seven isolates of PRSV-P as compared to healthy leaves. The content of organic carbon, nitrogen, phosphorus and potassium in PRSV-P infected papaya leaves were ranged 31.44-36.82, 3.25-3.99%, 3296.67-3736.67 ppm and 43.21-46.32 meq, respectively. The highest content of organic carbon was found in leaves infected with LD followed by FL, SM, MO, VC, CS and MM. Nitrogen and phosphorus content in leaves infected with LD and FL was statistically similar but significantly higher as compared to other isolates. The lowest content of nitrogen was found in leaves infected with MM, which was statistically similar to MO, VC and CS. Phosphorus content was minimal in leaves infected with MM followed by VC, CS, MO and SM. The content of potassium was significantly ($p = 0.05$) decreased in leaves infected with seven isolates of PRSV-P as compared to healthy leaves. Potassium contents in leaves infected with MO, FL, SM, LD and CS were statistically similar but significantly lower as compared to only MM (Table 2).

Table 2: Contents of cellular nutritional elements and nucleic acids obtained from papaya leaves with seven symptomatic isolates of PRSV-P and healthy leaves

	Cellular nutrient elements				Nucleic acids (mg g^{-1})	
Symptomatic isolates	Organic carbon (%)	Nitrogen (%)	Phosphorus (ppm)	Potassium (meq)	RNA	DNA
Mild mosaic	31.44^{d}	3.25^{c}	3296.67^{e}	46.32^{b}	30.25^{b}	9.66^{b}
Mosaic	34.50^{bc}	3.40^{c}	3501.11^{c}	44.79^{bc}	24.16^{c}	7.20^{c}
Fern leaf	36.32^{a}	3.97^{a}	3727.78^{a}	44.24^{c}	21.70^{d}	6.40^{c}
Severe mosaic	35.30^{ab}	3.68^{b}	3551.11^{b}	44.51^{c}	22.09^{d}	7.30^{c}
Vein clearing	33.60^{c}	3.34^{c}	3446.67^{d}	44.79^{bc}	22.00^{d}	6.80^{c}
Leaf distortion	36.82^{a}	3.99^{a}	3736.67^{a}	43.21^{c}	21.53^{d}	6.40^{c}
Chlorotic leaf spot	33.54^{c}	3.32^{c}	3453.33^{d}	44.80^{c}	21.54^{d}	6.90^{c}
Healthy	26.70^{e}	2.97^{d}	2874.44^{f}	50.28^{a}	35.48^{a}	12.46^{a}
CV (%)	4.98	7.58	1.33	3.29	8.47	7.24

Values within the same column with different letter(s) are significantly different at 5% level by DMRT

Table 3: Contents of cellular nutritional elements and nucleic acids obtained from papaya leaves at three different growth stage of papaya plant

	Cellular nutrient elements				Nucleic acids	
Plant growth stage	Organic carbon (%)	Nitrogen (%)	Phosphorus (ppm)	Potassium (meq)	RNA	DNA
Early	37.44[a]	3.91[a]	3637.08[a]	45.44[a]	21.95[b]	6.88[a]
Mid	33.10[b]	3.51[b]	3467.92[b]	45.32[a]	24.47[a]	7.03[a]
Late	30.02[c]	3.06[c]	3240.42[c]	45.34[a]	25.89[a]	7.30[a]
CV (%)	4.98	7.58	1.33	3.29	8.47	7.24

Values within the same column with different letter(s) are significantly different at 5% level by DMRT

Organic carbon, nitrogen and phosphorus content due to infection with seven symptomatic isolates of PRSV-P were recorded to be increase. The highest percentage of increase was found with LD infection in all cases. The minimum percentage of increase in organic carbon, nitrogen and phosphorus recorded with MM infection. Potassium content was decreased due to infection of different symptomatic isolates of PRSV-P. Reduction of potassium content was lowest in leaves showing the symptom of MM and highest with the symptomatic isolates of LD (Table 2).

Three growth stage of plant infected by different symptomatic isolates of PRSV-P differed significantly ($p = 0.05$) in respect of organic carbon, nitrogen and phosphorus content. Significantly, the highest content of all the three structural cellular components were obtained from leaves infected at early stage, which was followed by mid and late growth stage. Potassium content was not significantly different in varying plant growth stage infected by PRSV-P (Table 3).

Effect on Nucleic Acid Content

RNA and DNA content decreased in papaya leaves due to infection with seven symptomatic isolates of PRSV-P as compared to healthy leaves. The RNA: DNA in papaya leaves increased due to infection with isolates of PRSV-P. The lowest RNA content was recorded in papaya leaves infected with LD, which was statistically similar to CS, FL, SM and VC, but significantly lower as compared to MM and MO. Among the infected leaves, the maximum RNA content was recorded when infection was caused by MM followed by MO. The RNA content in leaves infected with MM and MO was significantly different (Table 2).

The content of DNA in papaya leaves infected with FL, LD, VC, CS, SM and MO was statistically similar. DNA content was maximal when leaves were infected with MM, which was significantly higher as compared to the rest of the isolates. The increase in ratio of RNA and DNA content as infected with PRSV-P were statistically similar among the seven symptomatic isolates. The rate of RNA and DNA reduction were minimum with MM infection and maximum with LD infection. Highest reduction of DNA was also observed in FL (Table 2).

Effect of plant growth stage infected with the seven isolates of PRSV-P on DNA and RNA: DNA found statistically insignificant. RNA content obtained from plant infected at late and mid stages of plant growth found statistically similar, but significantly higher than RNA obtained in plant infected with PRSV-P at early growth stage (Table 3).

Relationship of Papaya Yield with the Change of Cellular Contents

Fruit yield was negatively related with organic matter, nitrogen and phosphorus content as increased in PRSV-P infected leaves, but it was positively related with potassium content which is depleted in PRSV-P infected leaves. The relationship was linear having significant R^2 value except in case of nitrogen content where it was quadratic polynomial and also with significant R^2 value (Fig. 1).

Relationship of fruit yield of papaya with organic carbon ($R^2 = 0.8676$), nitrogen ($R^2 = 0.8299$), phosphorus ($R^2 = 0.867$) and potassium ($R^2 = 0.892$) could be described by the regression equation $y = -3.0957x_{oc} + 119.45$, $y = 42.622x^2_N - 325.21x_N + 626.69$, $y = -0.0366x_p + 141.87$ and

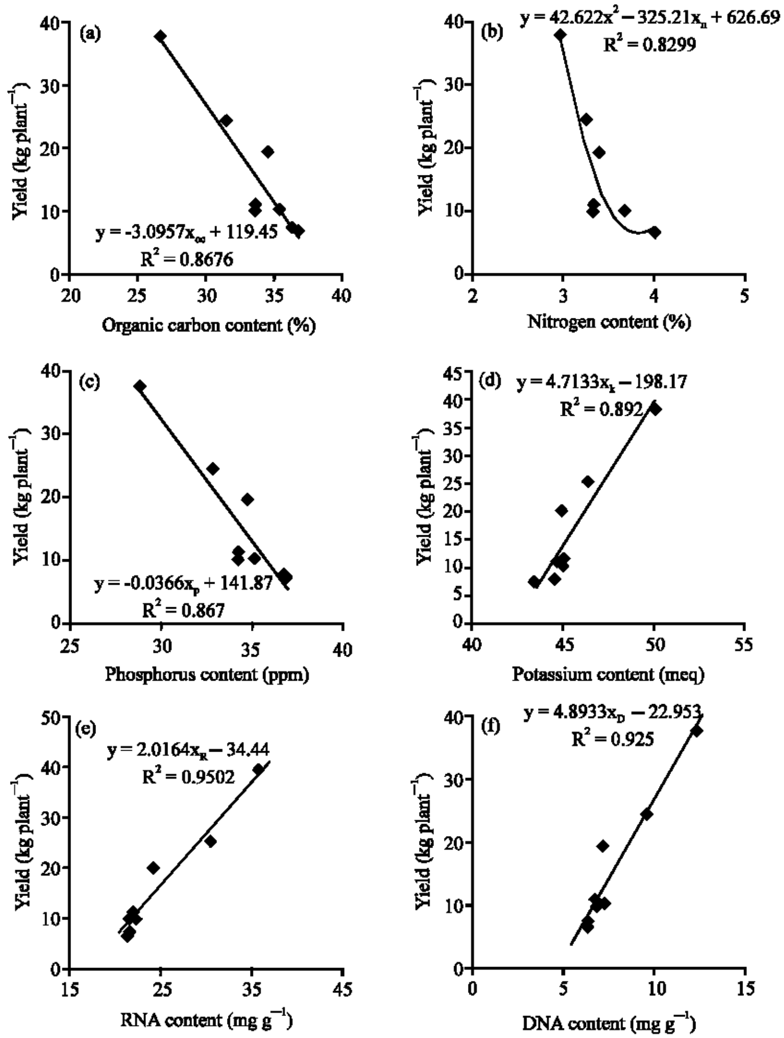

Fig. 1: Relationship of papaya yield with contents of different cellular nutritional elements (a) organic carbon, (b) nitrogen, (c) phosphorus, (d) potassium and nucleic acid (e) RNA and (f) DNA obtained from the plant infected with corresponding symptomatic isolates of PRSV-P

$y = 4.7133x_K - 198.17$, respectively, where y represents fruit yield and x_{oc}, x^2_N, x_p, x_K represent the content of organic carbon, nitrogen, phosphorus and potassium, respectively. All the regression equation expressed the relationship with significant R^2 value (Fig. 1).

Fruit yield of papaya was positively related with RNA and DNA content and these were decreased due to infection of the seven symptomatic isolates of PRSV-P. Relationship of fruit yield with the depletion of RNA ($R^2 = 0.9502$) and DNA ($R^2 = 0.925$) could be described by the regression equation as $y = 2.0164x_R - 34.44$ and $y = 4.8933x_D - 22.953$, respectively, where y represents fruit yield and x_R and x_D represent the value of RNA content and DNA content, respectively. In both relationships R^2-value was significant (Fig. 1).

DISCUSSION

The depletion or accumulation of cellular nutritional elements like organic carbon, nitrogen, phosphorus and potassium has been reported to be influenced by the host-virus specificity, which indicates that specific virus causes specific type of change in cellular components (Jensen, 1969; Watson and Watson, 1951; Matthews, 1991; Sarker *et al*., 1995). Increase of N content and decrease of phenol content in PRSV infected papaya leaves has been detected by Sarker *et al*. (1995) in Ranchi, India. Similar results were obtained by Akanda *et al*. (1998), Alam (1995), Alam *et al*. (1994, 1996), Hossain and Haider (1992) and Haider and Hossain (1994) in different host virus combination in Bangladesh. The results of the present study are in conformity with the reports of the previous workers mentioned earlier. Therefore, the results of the study with PRSV-P infecting papaya conclusively differentiated the identity of seven different symptomatic isolates of PRSV-P as distinctly different variants, each varied from the other in altering the cellular components in their respective infected papaya leaves.

The decrease amount of leaf chloroplast RNA synthesis and also cytoplasmic rRNA has been detected to occur in several host viral combination with symptoms of systematic mottling, mosaic or yellowing (Fraser, 1987; Hirai and Wildman, 1969; Mohamed and Randles, 1972). The authors mentioned earlier also detected the change in the amount of DNA in such host-virus interactions. It has generally been accepted that RNA viruses depleted the level of RNA and altered the level of DNA in the infected leaves (Atchison, 1973; Fraser, 1987; Goodman *et al*., 1986; Honeycutt and Millikan, 1964). The changes of nucleic acids so far bought by the viruses are highly virus specific (Fraser, 1987). Since the virus PRSV-P studied in the present experiment is a RNA virus. The alteration so far obtained in nucleic acids and in ratio of nucleic acids in the papaya leaves infected with seven different symptomatic isolates of PRSV-P seemed to be consistently varied depending on the variants identified on the basis of symptoms induced in papaya.

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