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## Alteration of Cellular Pigments of Papaya Leaves Infected with Seven Symptomatic Isolates of PRSV-P

<sup>1</sup>H. Rahman, <sup>2</sup>M.M. Alam, <sup>3</sup>S.B. Bhyan and <sup>4</sup>A.M. Akanda

<sup>1</sup>Department of Plant Pathology,

<sup>2</sup>Department of Genetics and Plant Breeding,  
Patuakhali Science and Technology University,  
Dumki, Patuakhali-8602, Bangladesh

<sup>3</sup>Department Agricultural Chemistry,  
Bangladesh Agricultural University, Mymensingh, Bangladesh

<sup>4</sup>Department of Plant Pathology,  
Bangabandhu Sheikh Mujibur Rahman Agricultural University,  
Salna, Gazipur, Bangladesh

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**Abstract:** The study was conducted to elucidate the diverse change in the amount of cellular pigment contents of papaya leaves infected with different biological variants of *Papaya ring spot virus*-Papaya strain (PRSV-P). Among the naturally prevailing PRSV-P strains in Bangladesh 7 different and distinctly defined ailments of symptoms, namely Mild mosaic, mosaic, fern leaf, severe mosaic, vein clearing, leaf distortion and chlorotic leaf spot has been selected to conduct the tests of this experimentation. Different partition of cellular pigments (Chlorophyll-a, Chlorophyll-b and  $\beta$ -carotene) were measured to quantify the alteration of cellular components. For every partition of pigments tested, the highest alteration was found in leaf bearing leaf distortion symptom. In most of the cases the lowest shift in pigment content was determined with the leaf showing Mild Mosaic symptom followed by the mosaic, severe mosaic, vein clearing, chlorotic leaf spot, fern leaf and leaf distortion symptom, respectively. The variability in all the partition of pigments contents alteration was found to be consistently correlated with 7 symptomatic variants of PRSV-P.

**Key words:** PRSV-P, symptomatic isolates, cellular pigments, partition, chlorophyll,  $\beta$ -carotene

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## INTRODUCTION

Viral infection results in the development of specific symptoms often comprise color aberration, which ultimately determines the extent of damage to the host plant and yield. The harvest loss from virus disease determined by the type and severity of symptoms observed on the foliar parts. The type of symptoms as well as the severity seem to be dependent upon nature of individual virus i.e. symptom is specific to viral pathogenicity; this is an intrinsic property of the virus (Bawden, 1959; Diener, 1963; Matthews, 1991; Porter, 1959; Sadasivan, 1963; Sing *et al.*, 1994). Generally color deviations like yellowing, mosaic, chlorosis etc. indicate effect of virus on pigment synthesis of infected host cell (Porter, 1959; Sadasivan, 1963; Zaitlin, 1987).

Radiant energy harvesting pigments in photosynthetic electron transport system affected by virus (Stahelin and Arntgen, 1983) and manifested as color deviations on infected plants parts are

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**Corresponding Author:** Dr. Habibur Rahman, Department of Plant Pathology,  
Patuakhali Science and Technology University, Dumki, Patuakhali-8602, Bangladesh

specifically due to either drastic reduction or cessation of chlorophyll synthesis, which ultimately affects the photosynthesis (Sreenivasulu *et al.*, 1989). Aberration in chlorophyll contents leads to the aberration in carotenoids particularly  $\beta$ -carotene content in virus infected plants (Mandhar and Singh, 1972).

*Papaya ring spot virus*, a member of the genus potyvirus, is non persistently transmitted by aphid to Papaya and members of the Chenopodiaceae and Cucurbitaceae families (Purcifull *et al.*, 1984). The PRSV strains that infect papaya, designated as PRSV-P, are serologically distinct from PRSV-W strains (water melon strain), which infect cucurbits (Purcifull *et al.*, 1984; Quiot-Douine *et al.*, 1986; Yeh *et al.*, 1984). Bateson *et al.* (1994) commented that PRSV-P might be originated through a mutation of the PRSV-W. The biological variability of virus and exerted distinct effect of variants on host like other entities has long been established. The biological variability of PRSV-P has been studied and the existence of variants in this virus was recognized by Quiot-Douine *et al.* (1990) in Morocco, Davis *et al.* (1999) in Florida and Kuan *et al.* (1999) in Rachi, India. Symptomological variants of PRSV-P has been recognized by Kawano and Yonaha (1992), Cai and Fan (1994), Dahal *et al.* (1997), Brunt *et al.* (1997), Gonsalves (1998), Kiranmai *et al.* (1998) and Choy *et al.* (2001).

Cross protection using mild strain or avirulent strains of PRSV-P and development of transgenic papaya through introduction of PRSV-P coat protein gene seem to have bright prospect of PRSV-P management (Yeh and Gonsalves, 1984; Chiang *et al.*, 2001). The development of coat protein gene mediated resistance from the existing genetic variability of PRSV-P isolates has been identified by Davis *et al.* (1999).

Study on population diversity of PRSV-P in respect of biological variability, symptomological isolates and identification of natural mild mutant is prior important before launching any cross protection program or introducing any transgenic line for PRSV-P management. Existence of distinctly different symptomological isolates of PRSV-P in Bangladesh is natural and frequently prevailing, therefore, a project has been launched to study the population dynamics of PRSV-P in Bangladesh and present experimentation is a part of that project objected to quantify the significantly altered amount of cellular pigments due to infection by each of the different symptomatic isolates of PRSV-P separately. This will suppose to provide information about the existence of distinct variant and identifying the mild one in PRSV-P.

## MATERIALS AND METHODS

Papaya variety Shahi was grown at Bangabandhu Sheikh Mujibur Rahman Agricultural University (BSMRAU) Farm, Gazipur, Bangladesh in 2003. 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 (Rahman, 2003) were selected and tagged.

Leaf samples were collected from the healthy and infected plants from the similar position and similar age showing distinct symptoms to maintain the uniformity of the sample. Three samples were 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 *et al.* (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) when it was required. All pigment partition was measured from the both healthy and PRSV-P infected leaf samples. Chlorophyll content was determined by extraction with 80% acetone method as proposed by Witham *et al.* (1986).

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

Identifying name	Description of principle ailments
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.
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.

The amount of chlorophyll-a, Chlorophyll-b and chlorophyll-a + b Were calculated using the formula as follows in fresh weight basis.

$$\text{Chlorophyll-a (mg g}^{-1}\text{)} = [12.7 (\text{OD}_{663}) - 2.69 (\text{OD}_{645})]V/1000W$$

$$\text{Chlorophyll-b (mg g}^{-1}\text{)} = [22.9 (\text{OD}_{645}) - 4.68 (\text{OD}_{663})]V/1000W$$

$$\text{Chlorophyll-a + b (mg g}^{-1}\text{)} = [20.2 (\text{OD}_{645}) + 8.02 (\text{OD}_{663})] V/1000W$$

Where,

V = Volume of acetone-chlorophyll extract (mL)

W = Fresh weight of the leaf sample taken (g)

Content of  $\beta$ -carotene was estimated following the method of Shiraishi (1972) extracting with petroleum ether. From the petroleum ether extract,  $\beta$ -carotene was estimated by measuring absorbance at 451 nm against petroleum ether blank on fresh weight basis.

$$\beta\text{-carotene (mg g}^{-1}\text{)} = 3.984 (\text{OD}_{451}) V/1000W$$

Where,

V = Final Volume of the petroleum ether- $\beta$ -carotene extract solution (mL)

W = Fresh weight of the leaf sample taken (g)

Statistical analysis was done using DMRT.

## RESULTS AND DISCUSSION

### Change of Cellular Pigment Contents

The contents of chlorophyll-a, chlorophyll-b and total chlorophyll were maximam in leaves of apparently healthy papaya plants. Contents of all types of chlorophyll were reduced significantly in leaves infected with all the seven symptomatic isolates of PRSV-P as compared to apparently healthy

leaves. The lowest reduction of all chlorophyll partitions was found in MM infected papaya leaves, which was followed by leaves infected with MO. The content of all measurement in chlorophyll with MM and MO differed significantly with each other and with the rest (Table 2). The lowest chlorophyll-a content was found in leaves showing LD symptoms. The content of chlorophyll-a in leaves possessing CS and VC symptoms was statistically similar but significantly ( $p = 0.05$ ) lower as compared to SM. The leaves carrying SM and LD symptoms had significantly different chlorophyll-a content. Significantly, the lowest chlorophyll-b content was found in infected leaves with LD symptoms. The content of the chlorophyll-b in infected leaves with SM, VC and CS symptoms was statistically similar but significantly higher as compared to only FL.

The total chlorophyll content was the lowest in leaves with LD symptom, which was followed by FL, CS, VC and SM symptoms, respectively. The adverse effect of VC and CS on total chlorophyll content was not significantly different but statistically similar in case of LD and FL symptoms. The total chlorophyll content was significantly higher in leaves showing SM symptoms than VC, CS, FL and LD symptoms but lower than MO and MM symptoms (Table 2).

The content of  $\beta$ -carotene as well as total pigment was maximal in leaves without viral infection symptoms (apparently healthy) of papaya. Both the parameters were significantly depleted due to infection with 7 different symptomatic isolates of PRSV-P. Like chlorophyll, the lowest reduction of  $\beta$ -carotene and total pigment contents was found in leaves infected with symptomatic isolates MM followed by that of MO isolate, the two isolates differed with each other and with the rest. The highest reduction of  $\beta$ -carotene was found in LD isolate infected leaves followed by VC, FL, SM and CS isolate, respectively. In case of total pigment content, maximum reduction was caused by LD symptomatic isolates infection, which was followed by FL, VC, CS, SM, MO and MM symptomatic isolates infection, respectively. The differences in total pigment content in leaves infected with seven symptomatic isolates of PRSV-P were significant (Table 2).

The ratio of chlorophyll-a and chlorophyll-b (chl-a:chl-b) was the highest in the leaves infected with symptomatic isolates MM followed by SM, VC, CS, LD, FL and MO infection. It was lowest in apparently healthy leaves of papaya. Among the 7 symptomatic isolates minimum chlorophyll a: chlorophyll-b was obtained with MO followed by FL and they were statistically similar. In SM and VC chlorophyll a: b were statistically similar but differed from the rest (Table 2).

The highest content of chlorophyll-a was found significantly ( $p = 0.05$ ) in papaya leaves collected at early stage of plant growth. The content of the pigment at mid and late stages of growth was statistically similar. The maximum contents of chlorophyll-b, total chlorophyll and total pigments were found at late stage followed by early and mid stage. The highest content of chlorophyll-a and the lowest content of  $\beta$ -carotene was recorded at early stage of plant growth and which get reversed at late stage. The differences of different pigment partition contents among the three stage of plant growth were significant (Table 3).

Table 2: Content of different pigment partition (Chl-a, Chl-b, Chl-a: Chl-b, Total chlorophyll,  $\beta$ -carotene and total pigments) in papaya leaves infected with seven symptomatic isolates of PRSV-P

Symptomatic isolates	Chlorophyll-a (mg/100 g)	Chlorophyll-b (mg/100 g)	Total chlorophyll (mg/100 g)	$\beta$ -carotene (mg/100 g)	Total pigments (mg/100 g)	Chl-a: Chl-b
Mild mosaic	43.53 <sup>b</sup>	19.64 <sup>b</sup>	63.17 <sup>b</sup>	16.22 <sup>b</sup>	79.39 <sup>b</sup>	2.22 <sup>a</sup>
Mosaic	28.50 <sup>f</sup>	15.71 <sup>c</sup>	44.21 <sup>c</sup>	10.84 <sup>f</sup>	55.05 <sup>c</sup>	1.81 <sup>cd</sup>
Fern leaf	22.20 <sup>f</sup>	11.77 <sup>e</sup>	33.97 <sup>f</sup>	8.39 <sup>ef</sup>	42.36 <sup>e</sup>	1.89 <sup>cd</sup>
Severe mosaic	27.48 <sup>d</sup>	13.56 <sup>d</sup>	41.04 <sup>d</sup>	9.17 <sup>de</sup>	50.21 <sup>d</sup>	2.03 <sup>b</sup>
Vein clearing	25.67 <sup>e</sup>	12.81 <sup>d</sup>	38.48 <sup>e</sup>	8.04 <sup>fe</sup>	46.52 <sup>f</sup>	2.00 <sup>b</sup>
Leaf distortion	20.28 <sup>g</sup>	10.65 <sup>f</sup>	30.93 <sup>e</sup>	7.23 <sup>e</sup>	38.16 <sup>b</sup>	1.90 <sup>cd</sup>
Chlorotic leafspot	25.25 <sup>e</sup>	13.19 <sup>d</sup>	38.44 <sup>e</sup>	9.92 <sup>e</sup>	48.36 <sup>e</sup>	1.91 <sup>bc</sup>
Healthy	40.49 <sup>a</sup>	29.22 <sup>a</sup>	69.71 <sup>a</sup>	20.85 <sup>a</sup>	90.56 <sup>a</sup>	1.39 <sup>d</sup>
CV (%)	2.80	5.07	2.46	8.28	2.69	6.32

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

Table 3: Content of different pigment partition (Chl-a, Chl-b, Chl-a: Chl-b, total chlorophyll,  $\beta$ -carotene and total pigments in papaya leaves infected with PRSV-P at three different growth stage of the plant

Plant growth stages	Chlorophyll-a (mg/100 g)	Chlorophyll-b (mg/100 g)	Total chlorophyll (mg/100 g)	$\beta$ -carotene (mg/100 g)	Total pigments (mg/100 g)	Chl-a: Chl-b
Early	39.13 <sup>a</sup>	17.41 <sup>a</sup>	56.54 <sup>a</sup>	10.29 <sup>c</sup>	66.83 <sup>a</sup>	2.25 <sup>a</sup>
Mid	27.57 <sup>b</sup>	15.65 <sup>b</sup>	43.23 <sup>b</sup>	11.33 <sup>b</sup>	54.55 <sup>b</sup>	1.76 <sup>b</sup>
Late	24.57 <sup>b</sup>	44.39 <sup>c</sup>	68.96 <sup>c</sup>	12.39 <sup>a</sup>	81.35 <sup>c</sup>	0.55 <sup>b</sup>
CV(%)	2.80	5.07	2.46	8.28	2.69	6.32

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

The degradation of chlorophyll or inhibition of chlorophyll production resulting the reduction of chlorophyll content in virus infected plants was reported by Esau (1956) and Peterson and Mckinney (1938). They concluded that the virus infected leaves have been attributed two causes-stimulation of chlorophyllase which degrades chlorophyll into chlorophyllide and phytol and inhibition of chloroplast development which leads to the development of colour deviations like mosaic, yellowing, chlorosis etc. Moreover, the interference in chlorophyll biosynthesis by the viruses has also been included into consideration by Granick and Beale (1983). Whatever might be the case, it is dependent upon the capability of virus itself in progressing pathogenesis in the host as reported by the authors as mentioned earlier. The reduction of total chlorophyll was 39% due to infection of *Bean yellow mosaic virus* in Beans (Chinnadurai and Nair, 1971), 63% in case of Okra infected by *Okra yellow vein mosaic virus* (Mandahar and Garg, 1972), 60% in case of Soyabean infected by *Soya bean yellow mosaic virus* (Narayanasamy and Palaniswami, 1973) and 36% in case of peanut infected by *Peanut green mosaic virus* (Naidu, 1984). Such variations were considered to be virus specific by the respective researcher.

The level of depletion of chlorophyll and  $\beta$ -carotene was found to be varied depending upon three different viruses infecting same variety of tomato as reported by Akanda *et al.* (1998) and Alam *et al.* (1994 and 1996). Similar observations were recorded by Hossain and Haider (1992) in case of country bean infected with country bean yellow vein mosaic virus and by Haider and Hossain (1994) in Okra due to infection of okra yellow vein mosaic virus.

The findings of the present study demonstrated that  $\beta$ -carotene reduction followed the similar trend as found in different partition of chlorophyll. The findings of the other scientists also support the results and trends of the present study (Mandahar and Singh, 1972; Akanda *et al.*, 1998; Alam *et al.*, 1994, 1996; Hossain and Haider, 1992; Haider and Hossain, 1994).

#### Relationship of Papaya Yield with Contents of Different Cellular Pigments

Yield of papaya fruit was linearly, positively and significantly related with chlorophyll-a, chlorophyll-b, total chlorophyll,  $\beta$ -carotene and total pigments. The relationship between fruit yield and chlorophyll a:b was quadratic polynomial (Fig. 1f). Relationship of fruit yield of papaya with chlorophyll-a ( $R^2 = 0.7814$ ), chlorophyll-b ( $R^2 = 0.9672$ ), total chlorophyll ( $R^2 = 0.9217$ ),  $\beta$ -carotene ( $R^2 = 0.9446$ ), total pigment ( $R^2 = 0.9348$ ) and chlorophyll a:b ( $R^2 = 0.7575$ ) could be expressed by the regression equation  $y = 1.1361x_a - 17.49$ ,  $y = 1.7457x_b - 11.96$ ,  $y = 0.7422x_{tc} - 17.439$ ,  $y = 2.2082x_p - 9.3697$ ,  $y = 0.5599x_{cp} - 15.883$  and  $y = 112.98x^2 - 426.43x_{ca:b} + 412.4$ , respectively, where y represents fruit yield and  $x_a$ ,  $x_b$ ,  $x_{tc}$ ,  $x_p$ ,  $x_{cp}$  and  $x_{ca:b}$  represent content of chlorophyll-a, chlorophyll-b, total chlorophyll,  $\beta$ -carotene, total pigment and chlorophyll a:b. In every relationship the  $R^2$ -value was found significant (Fig. 1).

The variability in all the partition of pigments contents alteration and the seven different symptomatic isolates of PRSV-P was found to be significantly interacted in respect of yield. Pigments contents alteration and yield is affected dependently upon the infection of different symptomatic isolates of PRSV-P. And dependency of the pigment availability was also found consistently correlated with seven symptomatic variants of PRSV-P as revealed from the significant value of co-efficient of determination ( $R^2$ ) obtained in all correlation and regression analysis employed in respect to the corresponding yield.

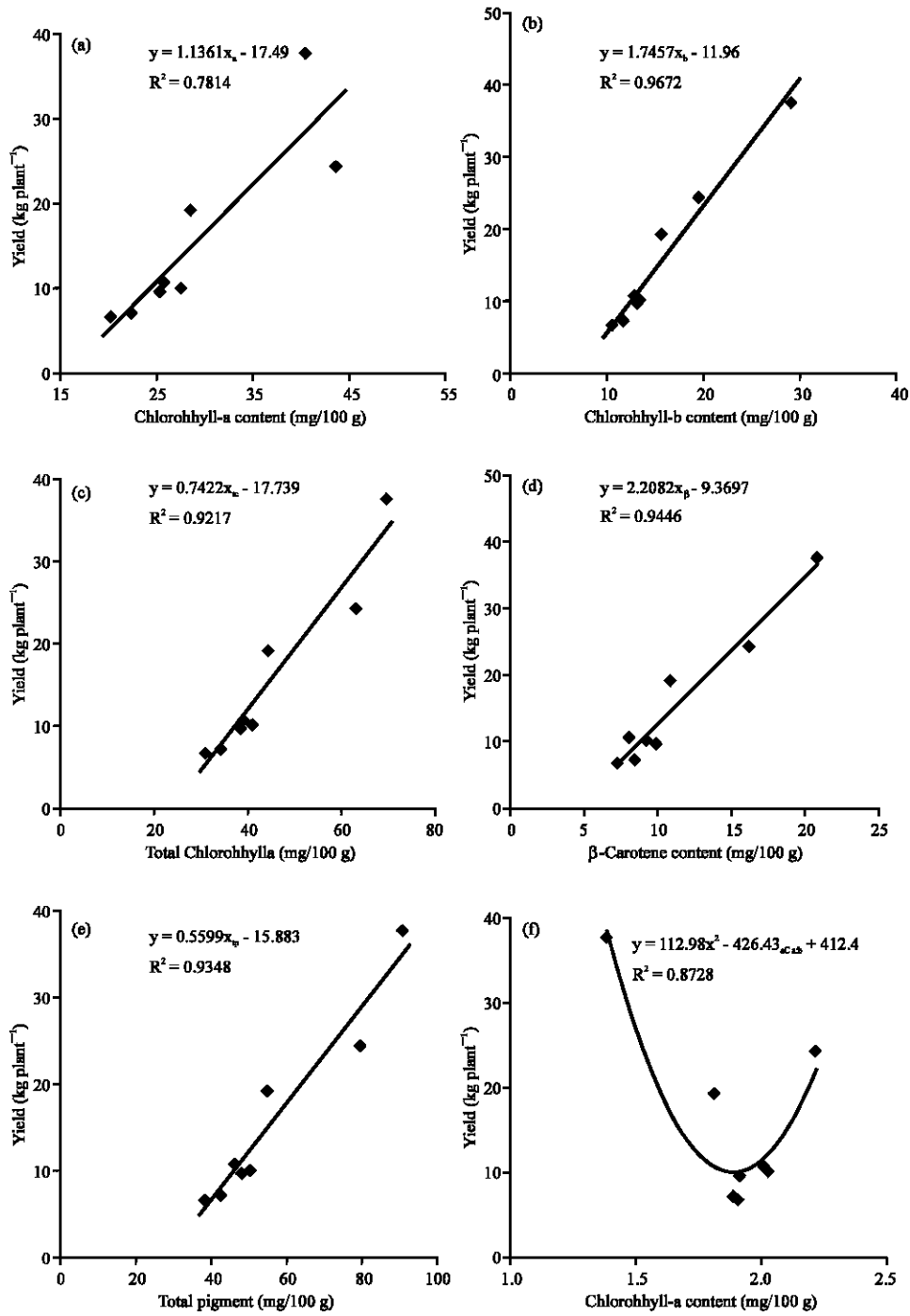


Fig. 1: Relationship of fruit yield of papaya with content of different partition of cellular pigments (a): chl-a, (b): chl-b, (c): total chl, (d):  $\beta$ -carotene, (e): total pigments and (f): chl-a:b, respectively) in papaya leaves infected

The results of the present study demonstrated that seven different symptomatic isolates of PRSV-P infecting papaya in Bangladesh might be the mutant strains of PRSV-P which distinctly differed in inducing the alternations of chlorophyll in their respective infected papaya leaves. The works of the other authors as mentioned earlier support the findings of the present study.

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