ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

# Biochemical Variability among Isolates of Citrus Yellow Mosaic Virus Infecting Different Species of Citrus in India

**Susmila Aparna Gaddam [1, 2], Venkata Subbaiah Kotakadi [2,3 *], DVR Sai Gopal [2,3] and MN Reddy[1]**

[1]Department of Applied Microbiology, S.P.M.V.V. Tirupati-517502 A.P. India.
[2]Department of Virology, S.V.University, Tirupati-517502. A.P. India
[3]DST-PURSE Centre, S.V.University, Tirupati-517502. A.P. India

## ABSTRACT

The yellow mosaic disease of citrus is one of the important diseases causing heavy losses in citrus industry. In the present study, an attempt has been made to analyze certain biochemical changes in citrus yellow mosaic virus infecting Rangpur lime sweet orange and acid lime. The virus disease of citrus was recognized by mosaic symptoms were noticed in sweet orange, rangapur lime and acid lime plants. It is widely distributed severe disease in India. Trees affected by the disease not only produce significantly lesser fruit both in number and in size. Systemically infected Rangpur lime, sweet orange and acid lime leaves showing characteristic yellow mosaic were collected from AICRP on Tropical Fruits (Citrus), Tirupati. The virus culture was maintained on citrus plants. Leaf samples were collected and processed for biochemical studies. Three individual sampling was than for each experiment. The fresh citrus leaf samples were collected and chlorophylls, proteins, sugars, starch, lipids, Ascorbic acid and total nucleic acids were estimated in both healthy and infected citrus plants. The total chlorophyll, starch and sugars, ascorbic acid contents were less in infected leaves compared to healthy. Total leaf proteins were more in mosaic virus infected leaves. Mosaic virus infected leaves showed higher total DNA and RNA contents than healthy leaves. The fresh and dry weight was less in mosaic virus infected samples..

***Corresponding author***

ISSN: 0975-8585

## INTRODUCTION

Citrus is considered to be one of the most remunerative fruit crops of India, having a lasting niche in the international trade and world finance. The most important commercial citrus in India is the mandarin orange followed by the sweet oranges and acid limes. Citrus crop has significant importance in fruit economy of the country and as the second largest industry in India with respect to area and third largest with respect to production, although India ranks sixth among top citrus producing countries of the world. Collective citrus fruits (sweet orange, mandarins, lemons and limes) have estimated production of 28.72 lakh tonnes from 3.86 lakh hectares. It is believed that most of the species under the genus citrus are native to tropical and sub-tropical regions of South East Asia, particularly China and India. During the last two decades, a number of virus and virus - like diseases have been recorded from citrus trees in India [36]. Amongst these diseases "Citrus mosaic" caused by citrus yellow mosaic virus (CYMV), a badna virus is widely distributed in India [29, 2, 3] which affects some of the important species grown in India.

## MATERIALS AND METHODS

### Estimation of Chlorophylls

Chlorophyll estimation was carried out according to the method of [4]. The young leaves of healthy and citrus yellow mosaic virus infected leaves (rangpur lime, sweet orange and acid lime) were collected, removed the mid ribs and cut into small pieces. One gram of both healthy and infected samples were taken separately, washed with tap water followed by the distilled water. The samples were macerated in cold 80% acetone and centrifuged at 3000 rpm for 15 min. The pellet was thoroughly washed thrice with 80% acetone and the supernatants were pooled. The pellets were discarded and pooled supernatants were made upto 25 ml with 80% acetone and color intensity read at 663 nm and 645 nm. The following formulae were used for estimation of total chlorophyll, chlorophyll a and chlorophyll b contents by Arnon,[4]

Total chlorophyll : $[(20.2 \times O.D_{645}) + (8.02 + O.D_{663})]$ df

Chlorophyll a : $[(12.7 \times O.D_{663}) - (2.69 + O.D_{645})]$ df

Chlorophyll b : $[(22.9 \times O.D_{645}) - (4.68 + O.D_{663})]$ df

Where O.D stands for optical density and d.f for dilution factor. The results were expressed as mg of chlorophyll / g fresh weight.

### Estimation of Total Sugars and Starch

The estimation of total sugars was done according to the method of Dubois et al [13] and total starch content by method of Mc Cready et al., [24]. 500 mg of healthy and infected leaves were taken, washed thoroughly with tap water followed by distilled water and blotted to dry in between filter paper folds. The midribs of leaf samples were removed, cut into bits and

macerated with 5 ml of 80% ethanol. The macerates were transferred to centrifuge tubes and centrifuged at 5000 rpm for 15 min. The pellet was washed thrice with 80% ethanol. The supernatants were pooled and made upto known volume with 80% ethanol. The samples were heated in water bath at 85°C until the alcohol was completely lost from the samples. The supernatants were pooled and used for estimation of sugars. The pellet was subsequently used for extraction and estimation of starch.

**Estimation of total Sugars**

20 ml of healthy and mosaic virus infected pooled supernatants were taken separately into the test tubes. One ml of distilled water and 4 ml of cold anthrone reagent were rapidly added to each tube, shaken well and incubated for 10 min on ice bath and cooled at room temperature. The blank was prepared by taking 1 ml of distilled water and 4 ml of cold anthrone reagent. The absorbance of the samples was read at 625 nm in a spectrophotometer. Amount of total sugars was estimated by using a standard curve prepared for D-glucose.

**Estimation of Starch**

The pellet which was collected from the above process was solubilised in 5 ml of 52% PCA and boiled at 80°C for 10 min. The solution was filtered through glass wool. The filtrate was measured and made up to 10 ml with PCA. 20 µl of healthy and infected sample extracts were taken separately, added 3 ml of distilled water and 5 ml of anthrone reagent and incubated for 10 min in ice bath. The absorbance of the samples was read at 625 nm in a Spectrophotometer. The amount of starch was calculated by using glucose standard curve.

**Estimation of total leaf lipids**

Total lipids were estimated from the leaves, according to the method of Hoppe and Heitefus [15]. The young leaves of healthy and citrus mosaic infected leaves were collected, removed the mid ribs and cut into small pieces. One gram of both healthy and citrus mosaic samples were taken separately, washed with tap water, followed by the distilled water and macerated with cold petroleum ether. The extracts were filtered through whatman No.1 filter paper into boiling test tubes. The debris from the filter papers were carefully collected using spatula into respective motors and again thoroughly macerated using cold petroleum ether. The extracts were filtered through the filter papers and filtrates are pooled together.

The gravimetric weighing bottles were thoroughly cleaned with detergent, dilute HCl followed by tap water and rinsed with distilled water. The bottles were dried in an oven at 60°C and the initial weight of the bottles with the lipids was recorded. The volume of the pooled extracts of healthy and infected samples was reduced by evaporation, either by heating on a boiling water or by keeping in dessicator. The concentrated lipid extracts were transferred into the weighing bottles and the weight was recorded. The bottles were kept in the dessicator for the evaporation of the solvents in vaccum. The weight of the weighing bottles with lipid samples was recorded periodically until constant weight is recorded. The weight of the lipids

was calculated by subtracting the initial weight of the empty bottles from the final weight. The content of the total lipids was expressed per gram weight of healthy and infected citrus mosaic leaf samples. The following formula was used for estimation of total lipids.

**Weight of empty weighing bottle = $W_1$g**

**Weight of empty weighing bottle + lipid = $W_2$g**

**Weight of lipid = ($W_2$ - $W_1$) g**

The content of total lipids was expressed per gram fresh weight of citrus leaves.

**Estimation of Total Proteins**

Total leaf protein content was estimated by the method of Lowry *et al.,* [18]. One gram of both healthy and mosaic infected citrus leaves (rangpur lime, sweet orange and acid lime) were taken, washed thoroughly and blotted to dry in between filter paper folds. The midrib of the leaves was removed, cut into bits and homogenized separately in a mortar at 4°C using the grinding buffer (0.1 M Tris HCl, pH 8.3; 0.5 M Sucrose and 0.5% 2-mercaptoethanol) at the rate of 2 ml/gm. The homogenate was squeezed through muslin cloth and centrifuged at 10,000 rpm for 10 min. The supernatants were collected separately, added equal volume of 20% trichloroacetic acid (TCA) to each sample and kept for 2 hours at 4°C. The TCA precipitate was collected by centrifugation at 10,000 rpm for 10 min. The pellet was washed twice with 5% TCA and thrice with ice cold solvent ether. The final protein pellet was dried under vacuum and solubilised in a minimal known volume of 0.1 N NaOH solutions. Insoluble material was removed by centrifugation at 8000 rpm for 10 min and the soluble protein in the supernatant was estimated according to Lowry *et al.,* [18] 20 µl of protein obtained from healthy and various infected samples were taken and to each sample added 5 ml of freshly prepared alkaline copper sulphate reagent. The samples were mixed well and the solution was allowed to stand for 10 min at room temperature. After 10 min incubation 0.5 ml of Folin phenol reagent was added to each sample and mixed thoroughly. After 30 min incubation the absorbance of the samples was read at 660 nm by using spectrophotometer. The amount of total leaf protein (mg/g fresh weight) was calculated by using bovine serum albumin (BSA) standard curve.

**Estimation of Ascorbic acid**

Ascorbic acid was estimated by colorimetric method of Sadasivam and Manickam, [37]. In Leaves One gram of both healthy and mosaic infected citrus leaves (rangpur lime, sweet orange and acid lime) were ground by using mortar and pestle in 25 ml of 4% oxalic acid solution. The extracts were centrifuged and collected the supernatants. In Fruits the healthy and mosaic infected fruits (rangpur lime, sweet orange and acid lime) were taken and their juice was extracted separately, centrifuged and collected the supernatants. 10 ml of both the supernatants (leaves and fruits) were transferred separately into conical flasks, added bromine water drop by drop with constant mixing and made up to 25 ml with 4% oxalic acid solution.

Similarly, 10 ml of ascorbic acid stock solution was converted into dehydroform by bromination. 0.1 ml of each brominated samples were pipetted out and made up to 3 ml by adding distilled water. One ml of DNPH reagent was added followed by 1 to 2 drops of thiourea to each tube. A blank was set as above with water in place of ascorbic acid solution. The contents of the tubes were mixed thoroughly and incubated at 37°C for 3 hours. After incubation the orange-red osazone cryslates formed were dissolved by adding 7 ml of 80% $H_2SO_4$. Absorbance at 540 nm was measured and calculated the ascorbic acid content in the samples by using ascorbic acid standard curve.

**Extraction and Estimation of total DNA (Rapid method)**

**A. Extraction of total DNA from leaves**

The total leaf DNA was extracted by a rapid method described by Dellaporta *et al.,* [11]. Both healthy and mosaic infected citrus leaves (rangpur lime, sweet orange and acid lime) were washed, blotted dry and cut into small pieces. 5 gm samples were macerated in mortar with extraction buffer by using a pestle. The extract was transferred to a 50 ml conical flask and kept at 65°C in a water bath for 15 min with intermittent gentle shaking. 5 ml of 5 M potassium acetate solution was added, mixed vigorously and incubated in ice for 20 min. The sample was centrifuged at 4000 rpm for 20 min. The supernatant was filtered through two layers of fine muslin cloth and collected the filtrate. 2/3 volume of isopropanol was added to the filtrate (2 ml isopropanol to 3 ml filtrate) and shaken the tubes. The tubes were incubated at -70°C for overnight to precipitate total DNA. The total DNA was pelleted by centrifuging at 10,000 rpm for 15 min at 4°C. The pellet was washed with ice cold 70% ethanol followed by absolute ethanol. The DNA pellet was dried under vacuum. The DNA pellet was suspended in minimal volume of TE buffer. 10 µl of RNase (100 µg/ml) was added to the DNA solution and incubated at room temperature for 30 min to remove RNA impurity.

**B. Estimation of total DNA in leaves**

The total DNA obtained from leaves was estimated by the method of Burton, [9]. Separate marked tubes containing 1ml, 2 ml and 3ml aliquots of the isolated DNA were prepared and dissolved in standard saline citrate solution. The samples were made upto 3 ml with distilled water, added 6 ml of diphenylamine reagent to each tube mixed well, heated in a boiling water bath for 10 min and then cooled. The absorbance of the samples was read at 600 nm. The concentration of DNA was calculated by using a standard graph.

**Extraction and Estimation of total RNA**

**A. Extraction of total RNA from leaves**

Total plant RNA was extracted by the method of Brawerman [8]. 5 g of the healthy and infected leaves were taken into a mortar and macerated with extraction buffer. The homogenate was centrifuged at 2000 rpm for 3 min. The supernatant was transferred into flask

and mixed with 0.1 volume of 10% SDS by stirring for 2-3 min. An equal volume of buffered phenol was added. The contents were separated by centrifuging at 2000 rpm for 5 min and the upper aqueous phase collected into a separate flask. The lower and interphase was again shaken with equal volume of extraction buffer for 5 min and centrifuged at 2000 rpm for 5 min. The second aqueous phase was combined with the first one and an equal volume of buffered phenol was added and mixed gently. After 5 min. the sample was centrifuged at 2000 rpm for 5 min. The extraction and centrifugation steps were repeated at least five times or until the interphase showed no proteins. Finally, the upper aqueous phase containing RNA was collected and to it added about 250 mM of NaCl. Two volumes of cold ethanol (96%) was added and left the flask overnight at -20°C for RNA precipitation. RNA was collected by centrifugation at 2000 rpm for 10 min. The pellet (RNA) was washed with 70% ethanol, ethanol: ether (1:1 v/v) and finally with ether. The pellet was dried gently in vacuum for a few minutes. The RNA was dissolved in elution buffer for further analysis by vortexing.

**B. Estimation of total RNA in leaves**

A standard RNA (50 µg RNA/ml) solution was prepared in ice chilled 10 mM, Tris acetate, 1 mM EDTA buffer pH 7.2. The isolated RNA was dissolved in the above buffer solution to an approximate conc. 50 µg/ml. A series of tubes containing 0.5 ml, 1ml, 1.5 ml and 3ml of isolated RNA; 0.5 ml, 1ml, 1.5ml and 3 ml of 50 µg standard RNA/ml were prepared. Each tube was made upto 3 ml with water. In addition, a blank containing 3 ml of water was taken. 6 ml of orcinol reagent and 0.4 ml of 6% alcoholic orcinol were added to each tube, shaken to mix the contents and then heated in a boiling water bath for 20 min. The tubes were cooled and read the absorbance at 660 mm against the blank. A standard curve was drawn using $A_{660}$ and the concentration of standard RNA. The amount of total RNA in the leaf samples was calculated by using the standard curve.

## RESULTS

**Estimation of Chlorophyll**

Citrus mosaic had reduced the total chlorophyll in mosaic infected citrus leaves of rangpur lime (-46.54)$^{b}$, sweet orange (-30.42$^{)b}$ and acid lime (-24)$^{b}$. While the decrease of chlorophyll a, chlorophyll b and chlorophyll a/b ratios over the corresponding health leaves was -30.42$^{b}$, -47.13$^{b}$ and -24$^{b}$ in rangpur lime; -33.95$^{b}$, -48.39$^{b}$ and -28.17$^{b}$ in sweet orange; and -45.56$^{b}$, -56.0$^{b}$ and -31.64$^{b}$ in acid lime. The results are presented in the table 5. The total chlorophyll were decreased in both mosaic infected sweet orange (-14.09)$^{c}$ and mosaic infected acid lime (-84.12)$^{c}$ leaves over mosaic infected rangpur lime leaves. In mosaic infected sweet orange leaves, Chlorophyll a (-1.99)$^{c}$ and chlorophyll b (-44.9)$^{c}$ and in mosaic infected acid lime leaves, with chlorophyll a (-25.94)$^{c}$ and chlorophyll b (-60.47)$^{c}$ were decreased but the chlorophyll a/b ratio were increased in both mosaic infected sweet orange (+52.89)$^{c}$ and in mosaic infected acid lime (+47.43)$^{c}$ leaves over rangpur lime (control) leaves.(Table.1)

**Total sugars and starch**

The results of the estimation of total sugars and starch in healthy and different mosaic infected leaves of citrus are presented in Table 6. The total sugars were decreased in mosaic infected rangpur lime (-16.07)b, sweet orange (-15.51)b and acid lime (-17.14)b leaves when compared to the corresponding healthy leaves. Further the percentage of total sugar content was reduced in mosaic infected sweet orange (-3.96)c and increased in mosaic infected acid lime (+11.49)c over mosaic infected rangpur lime leaves. The total starch contents were also decreased in mosaic infected rangpur lime (-31.01)$^{b}$, sweet orange (-27.4)$^{b}$ and acid lime (-30.62)$^{b}$ leaves over corresponding healthy leaves. The starch content in infected sweet orange was decreased (-6.76)$^{c}$ and in infected acid lime it was increased (+15.46)$^{c}$ over infected rangpur lime leaves.(Table.2)

**Total lipids**

The change of total leaf lipids in healthy and infected citrus leaves were estimated and the results are summarised in Table 7. The total leaf lipids were decreased in mosaic infected rangpur lime (-16.6)$^{b}$; sweet orange (-18.49)$^{b}$ and acid lime (-13.68)$^{b}$ leaves when compared to healthy leaves. The total leaf lipid content was were increased in mosaic infected sweet orange (+4.87)$^{c}$ and decreased in mosaic infected acid lime (-7.47)$^{c}$ over mosaic infected rangpur lime leaves.(Table.3)

**Total protein**

The infected plant showed a rapid increase in the quantity of total leaf proteins in mosaic infected rangpur lime (+43.48)$^{b}$, sweet orange (+63.22)$^{b}$ and acid lime (+40.57)$^{b}$ as compared with healthy leaves. Further the percentage of total leaf protein was decreased by (-42.89)$^{c}$ in mosaic infected sweet orange and increased by (+20.76)$^{c}$ in mosaic infected acid lime over mosaic infected rangpur lime leaves as shown in the Table 4.

**Ascorbic acid:**

The total ascorbic acid content was decreased in virus infected leaves of rangpur lime (-24.8)$^{b}$, sweet orange (-48.33)$^{b}$ and acid lime (-24.69)$^{b}$ and fruits of rangpur lime (-6.25)$^{b}$, sweet orange (-9.09)$^{b}$ and acid lime (-8.33)$^{b}$ when compared to corresponding healthy leaves and fruits. The results are presented in the Table 9. The total ascorbic acid content was increased in both mosaic infected sweet orange leaves (+65.95)$^{c}$ and fruits (+16.6)$^{c}$ and decreased in both mosaic infected acid lime leaves (-112.7)$^{c}$ and fruits (-11.0)$^{c}$ over mosaic infected rangpur lime leaves and fruits.(Table 5)

ISSN: 0975-8585

**Table 1.Total chlorophyll, chlorophyll a, chlorophyll b, and chlorophyll a/b ratios in healthy and virus infected citrus leaves**

| Sample | Chlorophyll mg/g fresh leaves | | | |
|---|---|---|---|---|
| | Total chlorophyll | Chlorophyll a | Chlorophyll b | Chlorophyll a/b ratio |
| **Healthy** | | | | |
| 1. Rangpur lime | 1.5921[a] | 0.7201 | 0.4010 | 1.7950 |
| 2. Sweet orange | 1.6904[a] | 0.7752 | 0.8952 | 0.8660 |
| 3. Acid lime | 2.3492[a] | 1.1751 | 1.1732 | 1.0 |
| **Mosaic** | | | | |
| 1. Rangpur lime | 0.8511 (-46.54)[b] | 0.5010 (-30.42)[b] | 0.212 (-47.13)[b] | 2.3630 (-24)[b] |
| 2. Sweet orange | 0.9744 (-42.35)[b] (-14.09)[c] | 0.512 (-33.95)[b] (-1.99)[c] | 0.4622 (-48.39)[b] (-49.9)[c] | 1.1083 (-28.17)[b] (+52.89)[c] |
| 3. Acid lime | 1.568 (-50.8)[b] (-84.12)[c] | 0.6408 (-45.56)[b] (-25.94)[c] | 0.516 (-56.0)[b] (-60.47)[c] | 1.24 (-31.64)[b] (+47.43)[c] |

[a] values given are an average of three individual samples.[b] figures in parentheses indicate percent decrease (-) over healthy samples.[c] figures in parentheses indicate percent increase (+) and decrease (-) over rangpur lime infected samples.

**Table 2. Determination of Sugars and Starch in healthy and virus infected leaves**

| **Sample** | | **Sugar / Starch content mg / g fresh leaves** |
|---|---|---|
| **Sugars** | | |
| **Healthy** | | |
| | Rangpur lime | 36.7[a] |
| | Sweet orange | 37.9[a] |
| | Acid lime | 32.9[a] |
| **Mosaic** | | |
| | Rangpur lime | 30.8(-16.07)[b] |
| | Sweet orange | 32.02(-15.51)[b](-3.96)[c] |
| | Acid lime | 27.26 (-17.14)[b] (+11.49)[c] |
| **Starch** | | |
| **Healthy** | | |
| | Rangpur lime | 34.5[a] |
| | Sweet orange | 35.0[a] |
| | Acid lime | 29.0[a] |
| **Mosaic** | | |
| | Rangpur lime | 23.8(-31.01)[b] |
| | Sweet orange | 25.41(-27.4)[b](-6.76)[c] |
| | Acid lime | 20.12(-30.62)[b](+15.46)[c] |

[a]. values given are an average of three individual samples. [b]. Figures in parentheses indicate percent decrease (-) over healthy samples. [c] Figures in parentheses indicate percent increase (+) and decrease (-) over rangpur lime infected samples.

ISSN: 0975-8585

**Table 3. Determination of total lipids in healthy and virus infected leaves**

| Sample | Conc. of lipid mg/g fresh leaves |
|---|---|
| **Healthy** | |
| Rangpur lime | 30.0[a] |
| Sweet orange | 30.9[a] |
| Acid lime | 29.2[a] |
| **Mosaic** | |
| Rangpur lime | 25.02(-16.6)[b] |
| Sweet orange | 23.8 (-18.49)[b] (+4.87)[c] |
| Acid lime | 25.67 (-13.68)[b](-7.47)[c] |

[a].Values given are an average of three individual samples.
[b]. Figures in parentheses indicate percent decrease (-) over healthy samples.
[c] Figures in parentheses indicate percent increase (+) and decrease (-) over rangpur lime infected samples.

**Table 4. Determination of total proteins in healthy and virus infected leaves**

| Sample | Conc. of Protein mg/g fresh leaves |
|---|---|
| **Healthy** | |
| Rangpur lime | 11.98[a] |
| Sweet orange | 12.1[a] |
| Acid lime | 11.51[a] |
| **Mosaic** | |
| Rangpur lime | 17.19(+43.48)[b] |
| Sweet orange | 19.75(+63.22)[b](-14.89)[c] |
| Acid lime | 16.18(+40.57)[b] (+20.76)[c] |

[a].Values given are an average of three individual samples.
[b]. Figures in parentheses indicate percent increase (+) over healthy samples.
[c] Figures in parentheses indicate percent increase (+) and decrease (-) over rangpur lime infected samples.

ISSN: 0975-8585

**Table 5. Determination of Ascorbic acid in healthy and virus infected leaves and fruits**

| Sample | Conc. of Ascorbic acid | |
|---|---|---|
| | Leaves (µg/g) | Fruits (µg/ml) |
| **Healthy** | | |
| Rangpur lime | 0.625[a] | 64[a] |
| Sweet orange | 0.30[a] | 55[a] |
| Acid lime | 0.81[a] | 72[a] |
| **Mosaic** | | |
| Rangpur lime | 0.47 (-24.8)[b] | 60(-6.25)[b] |
| Sweet orange | 0.155(-48.33)[b](+65.95)[c] | 50(-9.09)[b](+16.6)[c] |
| Acid lime | 0.69(-24.69)[b](-112.7)[c] | 66(-8.33)[b](-10.0)[c] |

a. values given are an average of three individual samples
b. Figures in parentheses indicate percent decrease ( - ) over healthy samples.
[c] Figures in parentheses indicate percent increase (+) and decrease (-) over rangpur lime infected samples.

**Table 6. Determination of total DNA content in healthy and virus infected leaves**

| Sample | Total DNA content (µg/g) |
|---|---|
| **Healthy** | |
| Rangpur lime | 315[a] |
| Sweet orange | 340[a] |
| Acid lime | 297[a] |
| **Mosaic** | |
| Rangpur lime | 1300(+312.69)[b] |
| Sweet orange | 1450(+362.47)[b](-11.53)[c] |
| Acid lime | 1125(+278.78)[b](+13.4)[c] |

a Values given are an average of three individual samples
b. Figures in parenthesis indicate percent of increase (+) over healthy samples
[c.] Figures in parentheses indicate percent increase (+) and decrease (-) over rangpur lime infected samples.

ISSN: 0975-8585

**Table 7. Determination of total RNA content in healthy and virus infected leaves**

| Sample | Total RNA content (µg/g) |
|---|---|
| **Healthy** | |
| Rangpur lime | 400[a] |
| Sweet orange | 410[a] |
| Acid lime | 380[a] |
| **Mosaic** | |
| Rangpur lime | 600(+50.0)[b] |
| Sweet orange | 635(+54.87)[b](-5.83)[c] |
| Acid lime | 560(+47.36)[b](+6.63)[c] |

[a] Values given are an average of three samples
[b] Figures in parentheses indicate percent of increase (+) over healthy samples.
[c] Figures in parentheses indicate percent increase (+) and decrease (-) over rangpur lime infected samples.

## Nucleic acids

### Deoxy ribonucleic acid

In mosaic infected leaves of rangpur lime (+312.69)[b], sweet orange (+352.47)[b] and acid lime (+278.78)[b] had higher quantities of total DNA has compared to healthy leaves. The results are presented in Table 10. The total DNA content was decreased in mosaic infected sweet orange (-11.53)[c] and increased in mosaic infected acid lime (+13.46)[c] leaves over mosaic infected rangpur lime leaves. (Table.6)

### Ribonucleic acid

The total RNA content was enhanced in infected leaves of rangpur lime (+50.0)[b], sweet orange (+54.87)[b] and acid lime (+47.36)[b] when compared to healthy leaves. The results are presented in Table 11. The total RNA content was also decreased in mosaic infected sweet orange (-5.83)[c] and increased in mosaic infected acid lime (+6.66)[c] leaves over mosaic infected rangpur lime leaves.(Table 7)

## DISCUSSION

The physiology of virus infected plant tissues is of interest to understand the processes involved in the development of symptoms. Some of them involve pigments, carbohydrates, proteins, lipids, ascorbic acid and nucleic acid metabolism in tissues infected by a virus. There

are several reports in the literature indicating many changes in the physiology and biochemistry of host plants as a consequence of disease. Many investigators [23] in different host virus combinations have estimated and correlated the virus concentration to altered metabolic processes and symptom production and severity. In virus infected plant, production of chlorophyll may cease (chlorosis, necrosis), cell may either grow and divide rapidly or may grow very slowly and be unable to divide (distorsion, stunting) [1].

The changes in chlorophyll may affect the growth and yield of the plants. Virus induced symptoms involved changes in leaf pigmentation. Reduced chlorophyll content in virus infected plants is attributed to stimulation of normal cell enzymes like chlorophyllase that degrades chlorophyll, and utilization of plastid proteins or their precursors for the synthesis of virus protein. The reduction in chlorophyll might be due to the increased activity of chlorophyllase [14]. Altered ratios of chlorophyll due to virus have been observed in different virus - host interactions [40]. Other reasons for the decrease in chlorophyll content following virus infection may be accumulation of carbohydrates in the leaves. Watson [46] reported that spraying sugar beet yellows virus infected plants with sucrose increased the carbohydrate content of the sugar beet leaves and enhanced the development of chlorosis. Naidu *et al.* [30] have shown that the chlorophyll 'a/b' ratio was decreased at severe stage of infection mainly due to decreased chlorophyll 'a' levels in peanut green mosaic virus infected peanut leaves. Subsequent studies with improved techniques have shown that the chlorophyll 'a' associated with the reaction centre of photosystem II (i.e., CPa complex) was reduced to a greater extent than the antenna chlorophyll of light harvesting system (i.e., LHCPY, 1, 2, 3 and II d complexes). Changes in pigments are often considered to be the secondary effects on the host plant since many viruses appear to multiplying and accumulate in the cytoplasm of the cell. Although these changes appear to be secondary as far as synthesis is also is considered, they are an important part of the disease process, considering the plant as a whole [10].

In the present study host-virus system the total chlorophyll, chlorophyll 'a' and 'b' chlorophyll 'a/b' was decreased in mosaic infected leaves as compared to healthy leaves. The total chlorophyll, chlorophyll 'a', chlorophyll 'b' were decreased in mosaic infected sweet orange acid lime leaves over rangpur lime leaves but the chlorophyll 'a/b' ratios were increased in infected sweet orange and increased in mosaic infected acid lime over rangpur lime leaves. So, the simultaneous decrease in chlorophylls in systematically infected citrus leaves is similar to those of Narayanaswamy and Ramakrishnan [31], Bos [7], Mandahar and Garg [20]; Narayanaswamy and Palaniswamy [32], Crosbie and Matthews [10]; Sridhar *et al.* [43]; Murti [28]; Singh *et al.,* [41], Naidu *et al.* [30] and Bhavani *et al.* [6].

Influence of plant pathogenic viruses on the carbohydrate metabolism of the infected host is very important with regard to economic aspect of plant disease. Some viruses appear to have little effect on carbohydrates in the leaves, while others may alter both their rate of synthesis and rate of translocation. The decreased photosynthesis and increased respiration that occurs in virus- infected tissues are leading to altered metabolism of carbohydrates are characteristic. The decreased carbohydrates in virus infected host tissues was reported by [47, 31, 16, 17, 28, and 35]. Barley yellow dwarf virus (BYDV) infection alters the regulation of

carbohydrate metabolism and ultimately photosynthesis and also there by decreased starch production in seeds than uninfected barley cultivars. Viral infection often affects carbon assimilation and metabolism in host plants. Cucumber mosaic virus alters carbohydrate metabolism in melon plants. Source leaves infected with CMV are characterized by high concentration of sugars and low starch levels [12].

In the present investigation too the total carbohydrates (sugars and starch) were decreased in the mosaic infected rangpur lime, sweet orange and acid lime leaves when compared to healthy leaves. The starch and sugar contents were low in mosaic infected sweet orange and high in mosaic infected acid lime over mosaic infected rangpur lime leaves.

Chlorosis is a common symptom in virus diseases. Chloroplasts with their internal membranous extensions are rich in lipids and there is evidence showing the involvement of glycosyl glycerides in photosynthesis. This aspect of disease physiology received very little attention.

The present studies shows the total lipid / g wet of the tissue was reduced in systemically infected rangpur lime and sweet orange and acid lime leaves than healthy ones. The total leaf lipid content was slightly decreased in mosaic infected acid lime leaves but slightly enhanced in infected sweet orange leaves over mosaic infected rangpur lime leaves. Other investigations also showed correlations between decrease in chlorophyll content and concentrations of lipids in chlorotic tissues [15, 48] and Srinivasulu,[42]. So, in the present work, the observed decrease in total leaf lipids is similar to those of Sreenivasulu [42, Sudarsanamma [44], and Bhavani et al .,[6].

The total leaf lipids were increased in mosaic infected sweet orange and decrease in mosaic infected acid lime over mosaic infected rangpur lime leaves. The reduced content of lipids in virus infected leaves may be due to either lowered synthesis or enhanced degradation. [21]

Viral nucleic acid, the principal infective entry of the viral particle, directs its own and its characteristics protein synthesis. Virus infection may have a number of indirect effects on host protein metabolism. These could be related to the production of symptoms, to the possible competitive inhibition of plant growth by synthesis of significant amounts of a 'foreign protein' and to possible viral controls on expression of the host genome

The present studies amply substantiate the view that "virus infection of plants should be regarded as change in the protein metabolism of the host cells" (Bawden and Pierie, 1956). Imbalances in total leaf proteins were noticed in the mosaic infected citrus species. Significant increase in the total leaf proteins was noticed in the mosaic infected three species of citrus plants than healthy ones. Decreased quantities of total leaf proteins in mosaic infected sweet orange leaves and increased protein content in mosaic infected acid lime, when compared to mosaic infected rangpur lime leaves was noticed.

Increased levels of soluble proteins in some virus host plants were earlier reported by several workers in different hosts [34, 39, 28, 27, 35, 6 and 45 ] The higher protein content in virus infected plants is possibly due to the synthesis of virus coat protein and other virus associated non-structural proteins.

Murti [28] found an increase in protein in CYMV infected sweet orange leaves. This is expected since virus infection adds to the total and protein nitrogen, since non protein nitrogen is withdrawn from the host pool during virus synthesis. Hence, this is probably a reduction in non-protein nitrogen and an increase in total and protein nitrogen [28]. Martin *et al.* [22] reported two phases in the protein changes in the mosaic infected tobacco plants. According to them, in its first phase normal protein was decreased equal to an estimated amount of virus protein formed. It may, therefore, involve conversion of normal protein to viral nucleo - protein. In the next phase very rapid accumulation of virus protein occurred. This involves accelerated synthesis of normal or an increase in the supply of substrate for the synthesis of normal and viral protein.

Pirone and Davis[34] and Sheen and Lowe [39] observed that substantial increase in leaf soluble proteins in tobacco vein mottling virus (TVMV) infected tobacco leaves showing very mild mottling symptoms. Quantitative variation of soluble proteins in sub-cellular fractions was reported in tobacco leaves infected with TMV or TVMV [15]. Rao *et al.* [35] stated that the increased protein content in virus infected plants due to increased activity of RNA synthetase or RNA polymerase.

Significant increase in total leaf proteins noticed in the three species of mosaic infected citrus plants in the present experiment may, therefore, be interpreted to be triggered by the presence of' viral nucleo - protein in the host.

Ascorbic acid (AA) is an important organic acid controlling many phases of plant growth and development. Chloroplasts are suggested as the seats of synthesis [21]. Altered ascorbic acid level in virus diseased tissues is obvious as disturbed chloroplast metabolism in virus infected plants is well documented.

The studies on ascorbic acid content in mosaic infected leaves showed decreased ascorbic acid content in virus infected rangpur lime, sweet orange and acid lime than healthy leaves. The ascorbic acid content was also estimated in mosaic infected fruits. It was also decreased in mosaic infected rangpur lime, sweet orange and acid lime fruits than healthy fruits. Further, the analysis between infected leaves and fruits showed the ascorbic acid content in both leaves and fruits was increased in mosaic infected sweet orange and decreased in mosaic infected acid lime over mosaic infected rangpur lime leaves and fruits.

Ascorbic acid according to Mairold and Weber[19] was found to be less in albino leaves than in green leaves. Mapson [21] suggested that the chloroplasts were the seat of ascorbic acid synthesis, the altered metabolism of chloroplasts in virus infected leaves may affect the synthesis of ascorbic acid. Reduction in ascorbic acid content in virus - infected plants has also

been noticed by several workers [16, 41] suggesting that virus infection activated ascorbic acid oxidation is responsible for decreased ascorbic acid, besides suggested reduced availability of sugars for its synthesis.

In virus diseased plant physiology, nucleic acid metabolism assumes special attention as the infective part of the virus is itself a nucleic acid. Virus infection may have some affect on host cell DNA synthesis.

Little work has been carried out on host cell DNA synthesis during virus infection and replication. It is generally assumed that the viruses have little effect on host DNA synthesis and this needs unequivocal experimental support. Misawa *et al.* [26] observed a transitory increase in the nuclear DNA content in tobacco leaves at 4-10 hours after inoculation with CMV. Sastry and Nayudu [38] observed higher levels of RNA and DNA in TRSV - infected cowpea primary leaves.

Very few reports are available on DNA content of virus infected plants [26, 36, 5]. Host DNA synthesis is affected very little or its synthesis was normal in diseased plants.

The studies with CYMV, the total DNA & RNA contents were more in mosaic infected citrus leaves when compared to healthy leaves. Further, were the comparison with CYMV infected rangpur lime, sweet orange and acid lime showed that the total DNA and RNA contents were low in virus infected sathgudi leaves and high in virus infected acid lime leaves over mosaic virus infected rangpur lime leaves.

Plant viruses have evolved several strategies to ensure their successful replication within the susceptible plant cells. Early investigation revealed slight increased in total RNA following infection with TMV in tobacco leaves [23]. Many investigators have studied the effects of virus infection on ribosome level and ribosomal RNA synthesis, but the effects differed with virus - host interaction [23].

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