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Induced biochemical changes in the CMV infected cucurbit plants

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ABSTRACT

In present experimental studies are conducted to determaine the biochemical changes in cucurbita pepo due to infection of cucumber mosaic virus. Chlorophyll-a,b,total Chlorophylls, and DNA were found to be reduced in the infected leaves as compared to healthy ones. But total RNA, proteins and phenolics compounds were increased in the infected leaves. Due to the addition of viral proteins and RNA.

Key words: Induced biochemical changes, Cucumber mosaic virus (CMV), Cucurbits.

INTRODUCTION

Multiplication of virus particles in the infected plant cells alters biochemical compounds of cells such as chlorophyll, β-carotene, organic carbon, nitrogen, protein, phosphorus proteins, phenolic compounds and nucleic acids etc. (Fraser 1987). External manifestations of disease symptoms are the results of altered host metabolism. The extent of crop loss is mainly associated with severity of visible symptoms (Sreenivasulu *et al*. 1989). Greater understanding of these biochemical changes may increase the accuracy of disease loss assessment, which helps to know about the nature of the virus. The present study was undertaken to determine changes in concentration of chlorophylls,proteins,phenols and nucleic acid in cucurbita pepo due to infection of cucumber mosaic virus (CMV).

MATERIALS AND METHODS

Collection of CMV infected samples

Suspected virus infected plant samples of cucurbits and other field grown vegetable crops with severe yellow and Green Island of mosaic symptoms were collected from various agricultural fields of different localities and regions of cucurbit growing areas of in and around Tirupati.

Test plants:
Cucurbit plants with primary two leaf stage maintained in earthenware pots carefully maintaining in the laboratory conditions.

Inoculation of test plants:
Test plats were inoculated with CMV infected leaf sample by mechanical sap inoculation. Inoculam was prepared by leaves were deribbed and thoroughly washed with tap water and macerated by using 0.1 M phosphate buffer, pH 7.0 containing 1.0% sodium sulphite (1:2W/V) and 2- mercaptoethonol . The test plants were dusted with Carborundum (600 mesh) powder uniformly on the surface of the primary leaves of healthy pumpkin plant. A small piece of muslin soaked in freshly prepared virus inoculum and gently rubbed on the leaves. For control treatment carborundum dusted leaves were inoculated with phosphate buffer alone (Mock inoculation). The virus was maintained by periodical mechanical inoculation of healthy plants at primary leaf stage.

Chlorophyl estimation:
For estimation of Chlorophyll healthy and diseased leaves of 15, 30, 45, days were taken. The leaves were washed with distilled water and the water was soaked by fillter paper. Then, the leaves were de ribbed 100 mg were taken for grinding into mortar and pestle with 80% acetone. The ground solutions were taken into test tubes and the final volume made to 10 mL by adding 80% acetone. The solutions were centrifuged at 5000 rpm for 10 min. The supernatant was taken in clean test tubes separately and repeated the step for 2 times until the pellet is clear. The absorbance was recorded at 663 and 645 nm in a spectrophotometer. Chlorophyll a, b and total chlorophyll were calculated by using following formula

Chlorophyll a (mg g^{-1} tissue) = [12.7 (D663)-2.69 (D645)]xV / 1000xW
Chlorophyll b (mg g^{-1} tissue) = [22.9 (D645) - 4.68 (D663)]xV/1000xW
Total Chlorophyll (mg g^{-1} tissue) = [20.2 (D645) +8.02 (D663)]xV/1000 xW

Where:
D=Optical density at respective nm.
V=Final volume of chlorophyll extract in 80% acetone.
W=Fresh weight of the tissue extracted.

Estimation of Phenol content:
The phenol content was estimated using Folin-Ciocalteau reagent. 80% ethanol was used for extraction of phenols. One g plant material was ground in two 5 ml portions of 80% ethanol and centrifuged. The extracts were pooled and made up to 10ml. 0.1ml of ethanol extract was evaporated on a water bath, to which 6 ml water was added and shaken well before addition of 0.5mlFolin-Ciacalteau reagent. After 5 min, 2 ml of 20% sodium carbonate solution was added. After incubation for 30 min, absorbance at 660 ηm was measured. Using pyro catecholas standard, the phenol content in the leaf extract was calculated (Folin and Ciocalteau, 1927).

Estimation of Total protein content:
Total protein was estimated calorimetrically by using Bradford method (Bradford 1976) recording absorbance at 595 nm. Bovine serum albumin was used as standard. Protein content in leaf samples was recorded as µg of protein per g of leaf.

Estimation of Total Nucleic acid content:
Nucleic acid contents were estimated according to and Spirin (1958).

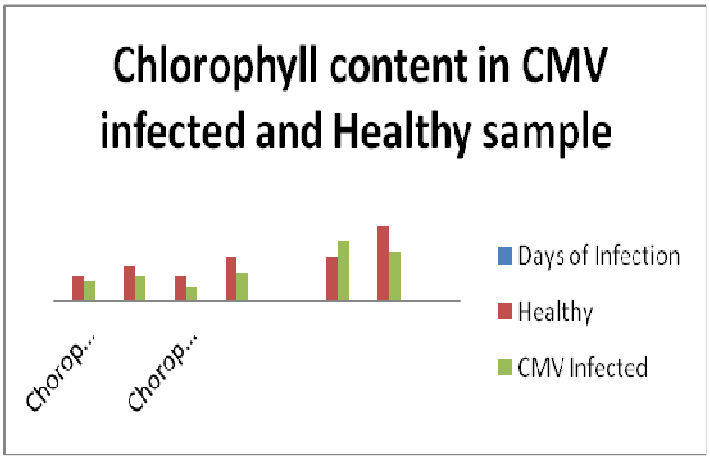

Fig.1. Comparison of chlorophyll a,b and total in CMV infected and healthy samples

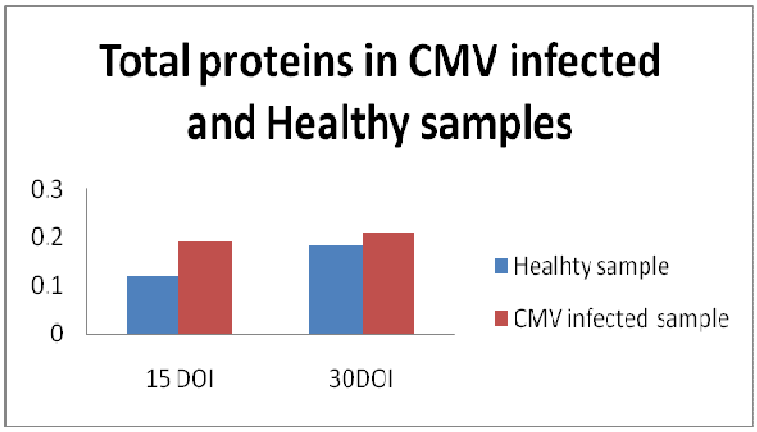

Fig.2. Comparison of total protein in CMV infected and healthy samples

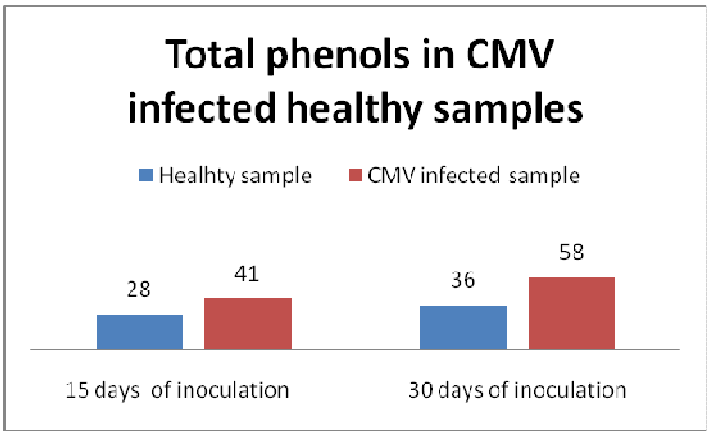

Fig.3. Comparison of total phenols in CMV infected and healthy samples

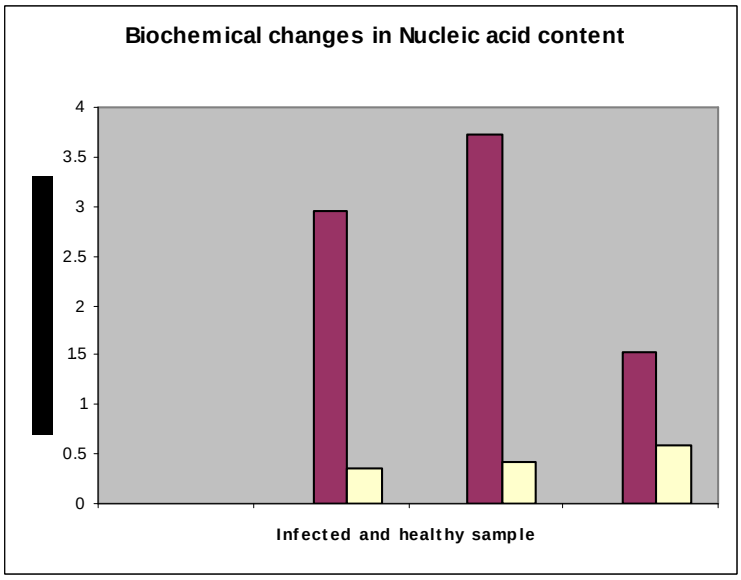

Fig.4.Changes in Nucleic acid content in CMV infected plants

RESULTS AND DISCUSSION

Chlorophyll content

Indicate a loss of total chlorophyll, chlorophyll a and chlorophyll b content due to virus infection in cucumber plants have been observed in the leaves of all varieties 15, 30, 45th day of infection. Chlorophyll a ranged from 0.04 to 0.09 in healthy Cucumber and in diseased it ranged 0.02 to 0.07 mg g^{-1}. Chlorophyll b ranged from 0.04 to 0.09 mg g^{-1} in healthy cucumber and in diseased it was 0.02 to 0.07 mg g^{-1}. Total chlorophyll in healthy were cucumber 0.09 to 0.18 mg g^{-1} and in diseased it was 0.03 to 0.12 mg g^{-1}.

Changes in total protein content in CMV infected plants*:*

There was a significant increase in the total protein content in plants treated with virus. In Healthy plants the protein content was 34 µg g-1 while in treated plants it varied from 36 to59 µg g-1. The maximum phenol content was observed in virus infected plants.

Changes in phenol content in CMV infected plants:

There was a significant increase in phenol content in plants treated though it was significantly higher (40 µg min-1 g-1) than that in the control (33 µg).

Changes in Nucleic acid content in CMV infected plants:

There was a significant increase in RNA content in plants.The ratio of RNA to DNA was increased in the infected leaves. Similar results were obtained by Hossain and Haider (1992). Higher content of RNA in the infected leaves might be due to predominant synthesis of viral RNA.

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