Journal of Phytopathology and Pest Management 3(1): 1-11, 2016
pISSN:2356-8577 eISSN: 2356-6507
Journal homepage: http://ppmj.net/

Changes in chlorophyll, phenols, sugars and mineral contents of cucumber plants infected with cucumber mosaic virus

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Abstract

Biochemical studies were made to monitor the relationship of chemical components and development of resistance in cucumber plants against *Cucumber mosaic virus* (CMV). Total soluble phenols, soluble sugars, chlorophyll and mineral nutrients; Potassium, Magnesium, Sodium and Lead were compared among resistant and susceptible varieties. Different metabolites showed different trends among diseased and healthy plants from resistant and susceptible groups of varieties. The phenolic and Magnesium contents increased in the inoculated plants of both susceptible and resistant genotypes. In resistant variety, rise in magnesium concentration in inoculated plants was less as compared to that of inoculated plants of the susceptible varieties. However, the chlorophyll, sugars, potassium were reduced in the inoculated plants of both reaction resistant and susceptible reaction groups. Plants from susceptible group presented a lower of level of nutrients as compared to un-inoculated ones whereas; the changes in sodium contents were not showing any relation to resistance mechanism.

Key words: Biochemical, CMV, resistance, chlorophyll, phenols, nutrients.

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Introduction

Cucumber mosaic virus (CMV) is among the most devastating and economically important plant viruses having approximately 85 families, 365 genera infecting 1200 monocotyledonous and dicotyledonous species in its host range (Roossinck, 2002). CMV is an aphid-transmitted virus belonging to genus *Cucomovirus*, family *Bromoviridae* that has caused severe losses since it has been first reported in 1916 (Ding et al., 1994; Palukaitis et al., 1992). CMV is a positive sense tripartite virus having three genomic single stranded RNA, which are encapsidated in a 28nm icosahedral particle separately (Nault, 1997). Proteins 1a and 2a are codded by RNA1 and RNA2, respectively those results in the formation of a replicase complex (Palukaitis et al., 1992). Replication of virus particles induces some alteration in the biochemical profile of the infected plant cells that imparts fluctuation in chlorophyll, phenolic compounds and nucleic acids etc. which is mainly related to the measure of crop losses (Muqit et al., 2007; Jagger, 1916). These biochemical changes impart the appearance of symptoms and severity of the disease is the measure of alteration in biochemical profile of the infected plant (Devi and Radha., 2012; Fraser, 1987). Objective of this study is to have some experiments for the better understanding of the fluctuations in the levels of chlorophyll contents, sugars and mineral nutrients associated with the resistance in *Cucumis sativus* against CMV.

Materials and methods

Source of CMV isolate and maintenance: Cucumber samples (*Cucumis sativus* L.) were collected from Rawalpindi region, Pakistan showing malformed, yellow-green mottled, vein banding, vein clearing on leaves. Samples were tested by double antibody sandwich ELISA (DAS-ELISA) as described by Clark and Adams (1977), to detect Squash mosaic virus (SqMV), Watermelon mosaic virus (WMV), and Zucchini yellow mosaic virus (ZYMV) and Cucumber mosaic virus (CMV). Based on DAS-ELISA results, CMV singly infected samples were selected as a source of virus for further experiments. Inoculum of the CMV isolate was prepared from freshly collected leaf samples of cucumber using 0.1 M phosphate buffer, pH 7.0 having 1.0% sodium sulphite (1:2W/V) and 2-mercaptoethonol, and was applied on leaves of selected host plants that were previously dusted with 600-mesh carborundum. After inoculation, the plants were maintained in a greenhouse at 25-30°C. Viral symptoms on cucumber plants were recorded three weeks after inoculation and then at regular intervals during the next four weeks. Symptomatic cucumber young leaves were collected from both inoculated and control plants three weeks post inoculation based on symptom expression and confirmed by ELISA test. Furthermore, chlorophyll, sugar and nutrient contents were estimated according to the following methods.

Chlorophyll measurement: Chlorophyll was extracted from leaves of both inoculated and control plants of all the cucumber genotypes by (Hiscox & Israelstam, 1979**)** using Dimethyl Sulphoxide (DMSO). 50mg leaf tissue was weighed and put in a test tube containing 7 ml DMSO preheated to 65°C in a water bath and boiled for 30-40 minutes. After discoloration of leaves the extracted liquid was transferred to a Falcon tube and the volume was made up to 10 mL with DMSO. Chlorophyll determination was done on spectrophotometer (Gensys) at wavelength 645nm and 663nm. Chlorophyll 'a' and 'b' as well as total chlorophyll were calculated following the equation used by Arnon, 1949.

Chl*a* (g l^{-1}) = 0.0127 *A*663 – 0.00269 *A*645;
Chl*b* (g l^{-1}) = 0.0229 *A*645 – 0.00468 *A*663;
Total Chl (g l^{-1}) = 0.0202 A663 + 0.00802 A645

Determination of total sugars: Total soluble sugars were determined in all the inoculated and healthy plants by the Anthrone reagent method (Yemm & Wills, 1954; Morris, 1948). 0.1g of fresh sample was taken from all the inoculated and control plants and macerated in 2-3 ml distilled water. The samples were heated in the water bath for 30 min at a temperature of 80^{o}C, samples were filtered, and the volume was made up to 5ml with distilled water and absorbance of the samples was measured instantly. 0.5ml volume of the sample was taken in the test tube and 5ml of Anthrone reagent was added to it and mixed carefully. The test tubes were placed in the water bath for 20 min at 80^{o}C. Afterwards, the absorbance of the samples was recorded in spectrophotometer at wavelength of 620nm. A glucose standard series was also run for comparison with the standard curve (0, 20, 40, 60, 80, 100 ug/ml).

Determination of phenols: The leave samples were collected from both inoculated and control cucumber plants for determination of soluble phenols (Julkunen-Titto, 1985). Sampling of fresh cucumber leaves was done after 30 days of inoculation. The samples were weighed individually (0.5g each) and then frozen. The frozen samples were powdered in liquid N with the help of Pestle and mortar. The powdered samples were scooped into 5 ml polystyrene tubes with caps. The samples were added with 2ml of 80% acetone and were placed in water bath for 1 hour at 50^{o}C for extraction. Supernatant from each sample was taken in microfuge tubes after centrifugation at 12000 rpm for 10 minutes. Fifty microlitre (50ul) of the extract was taken in a test tube of 10ml capacity, diluted to 1ml with distilled water, and then mixed with 0.5mL of 2 *M* Follin-Ciocateau's phenol reagent and 2.5 mL of 20% Na_2CO_3. The mixture was allowed to stand for 20 min at room temperature. Afterwards the absorbance of the samples was measured at 750nm using Hitachi (U-2001) spectrophotometer. The phenol concentration was measured from the standard curve prepared with Gallic acid.

Determination of nutrients: After drying the leaves in hot air oven at 70 ^{o}C the leaves were ground with the help of pestle and mortar. Twenty-five milligram

dried sample was boiled with 2.5ml of 1.4 N HNO3 on hotplate for 30 minutes. After cooling, the suspension was diluted 300 times with distilled water. Afterwards these samples were analyzed for the determination of nutrients including Potassium (K), Sodium (Na), Magnesium (Mg), and Lead (Pb) (Bhargava & Raghupathi, 1995). All the experiments were done keeping five replications in test and control plants.

Statistical analysis: The collected data was analyzed statistically using Analysis of Variance (ANOVA), comparison of mean values by LSD test and correlation techniques (Steel et al., 1997).

Results

Lead and chlorophyll measurement: Lead was determined in all the samples collected from inoculated and uninoculated plants of resistant and susceptible varieties. The level of Pb was found to be below detection level in all the samples. The results related to changes caused by CMV in chlorophyll contents of the resistant and susceptible genotypes are given in Figure 1. It is investigated that there is a significant difference in chlorophyll contents among inoculated and uninoculated plants. The resistant genotype Betaplha is not showing significant reduction in the chlorophyll contents as the uninoculated has 0.65g/l and the inoculated has 0.55g/l. However, all the susceptible genotypes are showing significant reduction in the chlorophyll contents in inoculated plants as compared to the uninoculated ones.

Sugars determination: The soluble sugars were recorded to be 341.05mg/Kg and 246.71mg/Kg in uninoculated and inoculated plants of the resistant variety; Betalpha. Similarly in the susceptible variety, babylone both uninoculated and inoculated had 600.22mg/Kg and 447.53mg/Kg sugar contents respectively. The results are shown in the Figure 2.

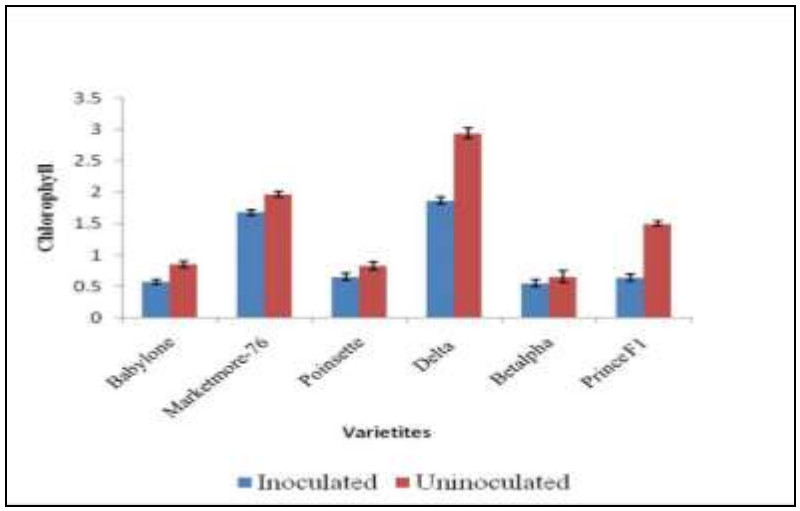

Figure 1: Comparison of chlorophyll contents among resistant and susceptible varieties of cucumber after infection with CMV. Bar in the column indicated the mean (+SD) of five plants in each replication.

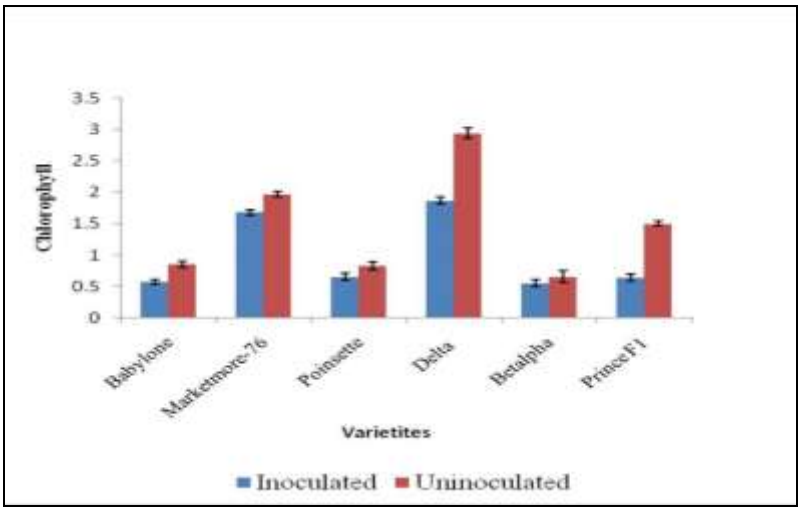

Figure 2: Comparison of soluble sugars among the resistant and susceptible cucumber varieties after infection with CMV. Bar in the column indicated the mean (+SD) of five plants in each replication.

Phenols determination: Although, all the inoculated plants of both resistant and susceptible plants had an increase in the phenolic contents after inoculation but the increase in the phenolic contents in the resistant variety is much more as compared to the susceptible varieties. The inoculated plants of resistant variety Betalpha have 118.79ug/g and the unicolated has 65.68ug/g. This increase is high as compared to the increase in the phenolic contents of the susceptible varieties that is shown in Figure 3.

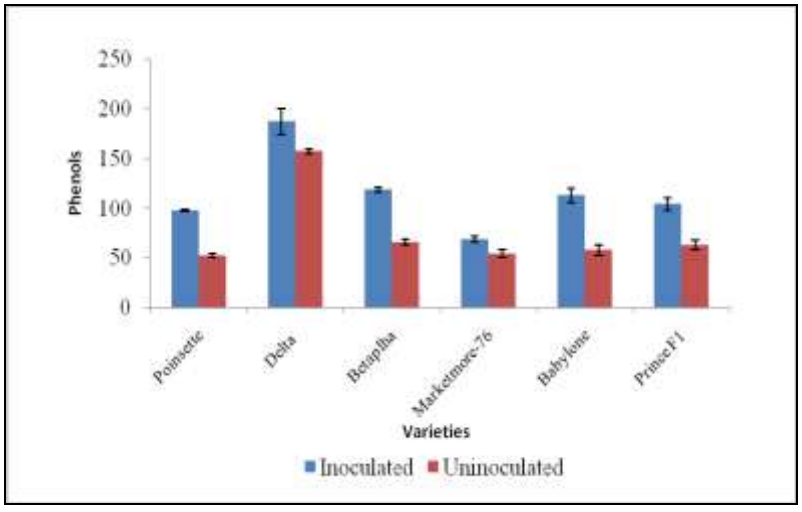

Figure 3: Comparison of phenolic changes among the resistant and susceptible cucumber varieties after infection with CMV. Bar in the column indicated the mean (+SD) of five plants in each replication.

Changes in mineral contents: Potassium was determined in the leaf samples of both inoculated and uninoculated plants of resistant and susceptible varieties and significant decrease was recorded upon inoculation. The data is compared in Figure 4. The present study revealed that viral infection causes a significant reduction in the plant potassium level as the resistant variety Betalpha inoculated had 2068.3 ppm and uninoculated had 2454.5 ppm. On the other hand, the susceptible variety Delta had 5399.0 ppm and 3464.5 ppm in uninoculated and inoculated respectively. Changes in the Magnesium contents are expressed in the Figure 5. It was concluded that all the samples of inoculated plants were having an increased level of Mg as compared to the uninoculated ones. Increment in the Mg contents of the resistant variety Betalpha was found to be highest concentration of Mg i.e. 2483.2 ppm dry weight in the inoculated plants and 2267.7 ppm dry weight in uninoculated whereas in the inoculated samples of susceptible variety Prince F1 the Mg concentration was 2064.4 ppm dry weight and uninoculated had 1731.7 ppm dry weight respectively. The comparison of means of Na in inoculated and uninoculated plants of resistant and susceptible genotypes is shown in Figure 6. Data shows that there is significant variation in Na concentration but is not related to the resistance mechanism. Comparison does not show a uniform response to infection in inoculated or uninoculated plants depicts that concentration of sodium is not related with resistance mechanism.

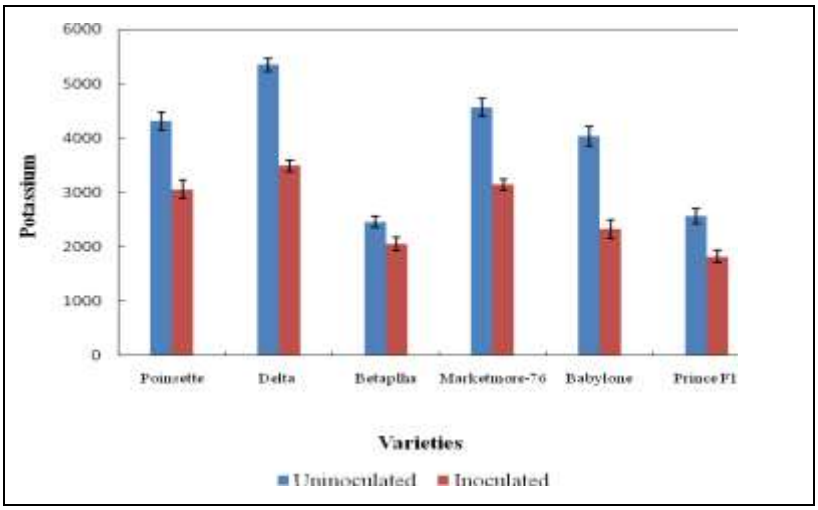

Figure 4: Comparison of potassium among the resistant and susceptible cucumber varieties after infection with CMV. Bar in the column indicated the mean (+SD) of five plants in each replication.

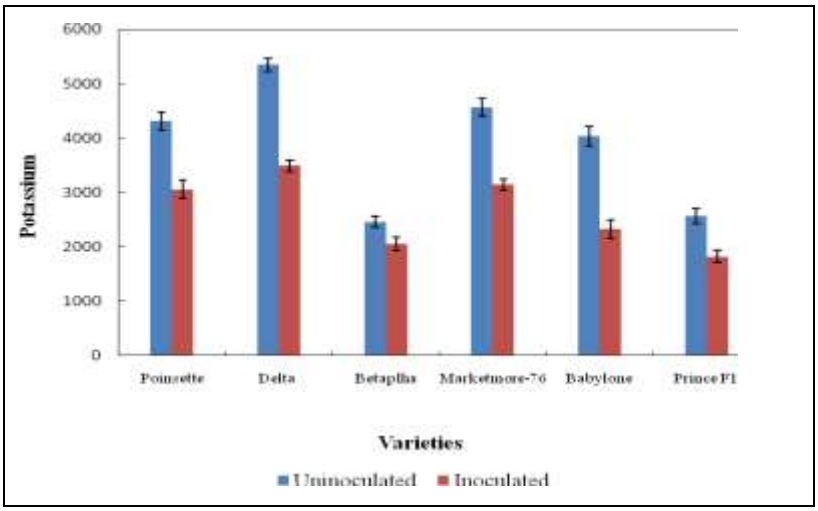

Figure 5: Comparison of magnesium contents among the resistant and susceptible cucumber varieties after infected with CMV. Bar in the column indicated the mean (+SD) of five plants in each replication.

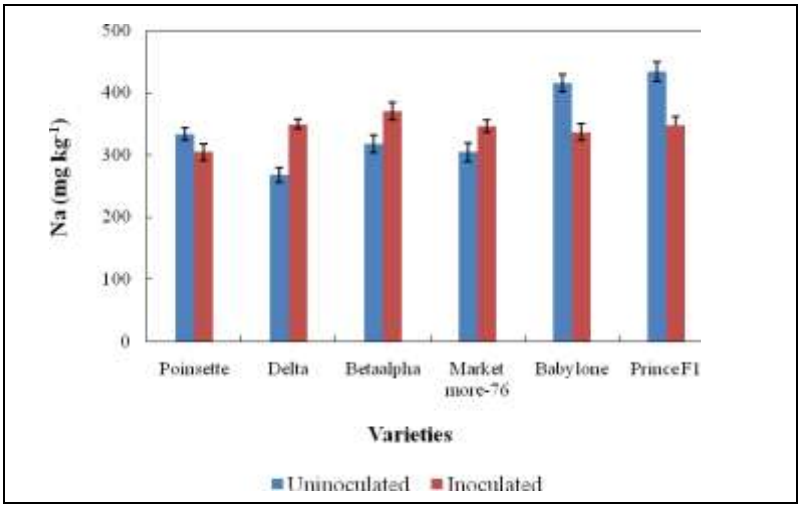

Figure 6: Comparison of sodium contents among the resistant and susceptible cucumber varieties after infected with CMV. Bar in the column indicated the mean (+SD) of five plants in each replication.

Discussion

Since, CMV was discovered for the first time (Doolittle, 1916), it has been detected in most countries and has posed itself to be a major threat to several host plants and resulted in epidemics. CMV is capable of infecting more than 800 plant species, making CMV one of the most important viruses for its economic impact. Development of resistant varieties is most effective way to avoid the disease losses. Different biochemical components have been found to exhibit variation in their concentration under the effect of virus infection. Chlorophyll contents were found to be reduced in all the plants either inoculated or uninoculated of both resistant and susceptible varieties but

reduction was recorded less in the resistant variety as compared to the susceptible one. Breakdown of chlorophyll leads to the development of mosaic symptom in the infected plants and lowers the photosynthetic rate. Reduction of photosynthetic rate causes depletion in the rate of sugar production in plant which causes loss of vigor. A healthy plant is comparatively more resistant to viral infection than a weak plant. It is necessary to save the chlorophyll contents of the plant to keep it resistant against the CMV infection as loss of this component will lead to the symptom development as well as loss of yield. The biochemical changes in the *Cucurbita pepo* L. due to CMV infection include starch accumulation. At early stages of infection, low starch contents were recorded however, this condition is reversed at later stages. The reduction in the soluble sugars is probably due to reduction in the chlorophyll contents, which leads to a reduced rate of photosynthesis eventually resulting in decline of total carbohydrate contents in the diseased plants (Radwan et al., 2007; Tecsi et al., 1994). *Cucumber green mottle mosaic tobamovirus* causes a significant reduction in the carbohydrate contents in leaf, stem and root samples after infection of cucumber (Sarivastava & Tiwari, 1998). As a result of virus infection, activity of the chloropyllase enzyme is enhanced multifold and causes degradation of the chlorophyll in the effected leaf tissue (Patel et al., 2013). CMV reduces the total chlorophyll contents more significantly in the susceptible genotypes as compared to the resistant ones as it is related to the typical symptom of CMV that is the mosaic pattern on the leaves (Nabila, 1999; Singh & Singh, 1991). Electron microscopy shown that the severe chlorosis induced after systemic infection with CMV-P6 lead to reduction in the size of chloroplasts that contained fewer grana (Roberts et al., 1982) and chlorophyll degradation can be used as a parameter to determine disease severity (Sandha et al., 1999). The Determination of lead was included in the study to confirm that the reduction in the chlorophyll contents is solely because of viral infection. As in previous studies, it was reported that Pb degrades the Photosynthetic pigments (Husain et al., 2006). The phenolic contents in the cucumber plants were significantly increased after the infection with CGMMV; mungbean plants infected with MYMV and in wheat plants infected with *Wheat streak sterility mosaic potyvirus* as compared to the healthy plants (Srivastava & Tiwari, 1998; Thind et al. 1996; Kofalvi & Nassuth 1995). The increase in the phenolic contents is supposed to be the first attempt by the plant to develop resistance against the virus (Nicholson & Hammerschmidt, 1992). Polyphenol oxidase converts phenols into quinone and its activity is enhanced in the systemically infected leaves because of accumulation of high levels of phenols. Phenol biosynthesis is regulated by Phenylalanine ammonia lyase (PAL) which is the key enzyme that works in the phenyl propanoid metabolism (Wen et al., 2005). Potassium is very important nutrient as it is mobile (Jones et al., 1991) and can be blocked in the phloem because of systemic infection by CMV. Potassium is involved in the enzymatic activity that is important for the carbohydrate metabolism and regulation

of stomatal opening that effects rate of photosynthesis (Salisbury & Ross, 1992). In the cucumber, leaves infected with CMV were having a low concentration of K, which increases the chances of establishment of virus (Jones et al., 1991). Magnesium plays an important role in manufacturing chlorophyll and consequently it is vital for the photosynthesis and carbohydrate metabolism (Devline & Witham, 1983). Magnesium was reported to be increased after inoculation in both resistant and susceptible varieties and this result referred to the increased photosynthesis (Reddy et al., 2005). Virus infection showed a significant reduction of Mg contents in susceptible genotypes in comparison to resistant genotypes. Mg concentration was found to be relatively higher in resistant varieties and showed very little increase after infection (Singh et al., 1998). The role of Na as a nutrient is very important but its role in the resistance mechanism could not be differentiated, as there is no specific trend of variation among inoculated and un-inoculated plants of resistant and susceptible groups of test plants. Data shows that concentration of Na is not related with resistance mechanism. The variation in the concentrations of Na in these varieties was probably the result of the varietal potential. Resistance to CMV in cucumber plants is an important attribute to harvest good product. The soils in which the cucumber plants are to be sown must be free from lead that can help us in avoiding the breakdown of chlorophyll contents of the leaves to avoid the symptom development. The extent of crop loss is mainly associated with severity of visible symptoms. Avoiding the symptom development can be helpful in maintaining crop production in acceptable range. Cucumber varieties having ability to enhance phenolic contents and produce more chlorophyll are able to maintain high photosynthetic rate and produce a higher level of sugar. These all collectively boost the potential of the plant for development of resistance against CMV.

Acknowledgements

The authors are grateful to the Plant Pathology Department, PMAS-Arid agriculture University Rawalpindi for providing research facilities and to Dr. Irfan ul Haque and Dr. Sardar Muhammad Mughal for cooperation and provision of the necessary facilities valuable for execution of this research. I am also thankful to my colleagues who assisted me in completing this research.

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