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METABOLIC CHANGES OF CUCUMBER PLANTS DUE TO TWO CMV EGYPTIAN ISOLATES

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ABSTRACT

Cucumber production exposed to huge losses in Egypt and worldwide due to infection with Cucumber mosaic Cucumovirus (CMV). The current study focus on the impact of two Egyptian CMV isolates on metabolic and oxidative activities in cucumber plants. Two CMV isolates was obtained from cucurbitaceous crops and confirmed by DAS-ELISA. Inoculated cucumber plants by infectious crude sap with two CMV isolates showed difference in external symptoms. The metabolic and biochemical components of cucumber plants were affected with two CMV isolates. The present study provided that, CMV infection caused a significant reduction in both photopigments and biochemical components while bioactive components (proline and phenol) and oxidative enzymes (POX, SOD, PPO and CAT) were increased significantly compared healthy ones. It was found that cucumber-CMV isolate was more effective in metabolic and biochemical than Squash-CMV isolate.

INTRODUCTION

Viral diseases dynamical varying problems and caused major economic losses (**Nameth et al 1986**). Cucumber mosaic virus considers one of the most important pathogens affecting crops worldwide.

CMV is worldwide amongst the five most important plant viruses, infecting vegetable and ornamental species (Cornelia Heinze et al 2012).

(Received 25 March, 2018) (Revised 28 March, 2018) (Accepted 1 April, 2018) CMV has one of the widest geographical deliveries of any plant virus, having been detected world-wide, both in tropical and moderate climates (Palukaitis et al 1992). CMV is one of the most destroyer and economically important plant viruses having nearly 365 genera, 85 families. The total harvest area for cucumber in Egypt up to 23046 Fadden in 2016 while was 20875 in 2014, and the production increased to 519858 ton in 2016 while was 473774 in 2014. (FAO, 2016).

CMV is a tripartite virus with abundant sequence variability; classified into three subgroups with 80 to 97% identical nucleotides in their coat protein (Cornelia Heinze et al 2012).

An affected biochemical changes in virus infected plants result in decrease of production quality of infected crops. Several investigators suggest that virus multiplication inside the plant cell alters biochemical ingredients of plants and disorder the physiological processes like transpiration, photosynthesis and respiration of the infected plants that affect the growth and crop (Tajul et al 2011; El-Dougdoug et al 2014a and Sofy et al 2017). Also, have informed that the determination of cellular ingredients in virus infected plant is very important to comprehend the activities of the host cell and the nature and degree of damage have caused by the virus. Viral infection stimulates increased absorbency in cells to cause loss of water. These also illustrate the cup shape of the infected leaves, mainly in the severe symptom stages (Oleinikova, 1969). Physiological processes important for plant activities and growth (Arfan et al 2011), and it is highly affected by viral infection (Radwan et al 2010; Sofy et al 2014 and Sofy et al 2017). Several reports suggest that virus multiplication inside the plant cell disorder the physiological processes like respiration, photosynthesis and transpiration, also changes different biochemical constituents of

the infected plants that affect the growth and yield (Tajul et al 2011; El-Dougdoug et al 2014b and Sofy et al 2017).

Carbohydrates, which mean one of the main organic components of the dry matter, derived from photosynthesis, were found to be affected by CMV and infected stress Moreover, a memorable reduction in the total protein was seen as a result of CMV infection. Infected cucumber plants accumulate more phenolic compounds as antioxidants to resist viral stress (EI-Dougdoug et al 2010, 2014 and Sofy et al 2018). The current study focus on the impact of two Egyptian CMV isolates on metabolic and oxidative activities in cucumber plants

MATERIALS AND METHODS

Source of cucumber plant and virus isolate

Seeds of *Cucumis sativus* L. alfa-beta plants were obtained from Agriculture Research Centre, Ministry of Agriculture, Giza, Egypt, and germinated in seed trays containing perlite. Seedlings were cultivated under natural lighting, day/night temperature of approx. 22/20°C and 60% mean relative humidity. One week old seedlings were potted in soil and grown under the same conditions.

Cucumber seedlings were checked before planted, were tested against CMV, *Watermelon Mosaic virus* (WMV), *Squash Mosaic virus* (SqMV), and *Cucumber Green Motel Virus* (CGMV) by DAS-ELISA technique using specific polyclonal antibodies (Clark and Adam, 1977).

Two CMV viruses were detected in naturally infected cucumber and squash plants cultivated in Gharbia and Sharqia Governorates, and identified biologically, serologically and molecularly previously (Farahat, 2017) (unpublished).

Experimental design

Certified seedlings and virus tested against (CMV, CGMV, SqMV, and WMV). Two plants per pot were planted and inoculated mechanically with CMV isolates at two cotyledons on leaves. Another two plants was set without CMV inoculation as a control for the two isolates. The plant samples were collected for analysis when the plants were 40 days old. The inoculated plants were kept under greenhouse conditions and examined for symptoms appearance. Serological detection was determined using CMV specific polyclonal antibodies by DAS-ELISA according to (Clark and Adam, 1977).

Metabolic Changes

Photopigmens constants

The method used for the quantitative determination of chlorophyll was that of **Vernon and Selly** (1966). The optical density of the plant extract was measured using spectrophotometer of two wave lengths (649, 665 and 470 nm) (Unico 2000).

Mg chlorophyll (a) / g tissue = 11.63(A665) - 2.39 (A649).

Mg chlorophyll (b) / g tissue = 20.11(A649) - 5.18(A665).

Mg chlorophyll (a + b) / g tissue = 6.45 (A665) +17.72(A649).

For carotenoids, equation: Car_{x+c} = 1000 ×(OD₄₇₀-1.82 C_a – 85.02 C_b)/ 198= mg/g fresh weight.

Total soluble carbohydrates: Total soluble carbohydrates were determined using anthrone techniques according to **(Umbriet et al 1969),** and measured (in terms of sucrose equivalents) using spectrophotometer at 620 nm (Unico 2000).

Phenolic compounds: Phenolic compounds in leaves were carried out according to the method described by **(Daniel and George, 1972)**. Using spectrophotometer (Unico 2000) at the wave length 725 nm.

Free proline: Free proline content was determined according to the method described by **(Bates et al 1973)** at 520 nm using UV- spectrophotometer (Unico 2000).

Total proteins: According to the method of **(Lowery et al 1951)** using casein as a standard protein determination of total protein in leaves by spectrophotometer (Unico2000) at the wave length 750 nm.

Antioxidant enzyme activates: were estimation according to (MuKherjee and Choudhuri, 1983).

Superoxide dismutase (SOD) activity: was determined by measuring the inhibition of the auto oxidation of pyrogallol using a method according to **(Marklund and Marklund, 1974)** at 325 nm using UV spectrophotometer (Unico2000).

Catalase (CAT) activity: was determined by measuring the rate change of H_2O_2 absorbance with a UV spectrophotometer (Unico2000) at 250 nm according to the method of **(Chen et al 2000).**

Peroxidase (POX) activity: was assayed using the rate of increase in absorbance as pyrogallol was determined by UV- spectrophotometer (Unico2000) at 470 nm (Bergmeyer, 1974).

Polyphenol oxidase (PPO) activity: was determined according to the method adopted by Matta and Dimond (1963). The absorbance was measured at 495 nm by UV spectrophotometer (Unico2000).

Statistical analysis:

All statistical calculations were done using SPSS (statistical package for the social science version 20.00) statistical program at 0.05 level of probability Snedecor and Cochran (1973). Quantitative data with parametric distribution were done using analysis of variance the two-way ANOVA and Post hoc-LSD tests (the least significant difference). The confidence interval was set to 95% and the margin of error accepted was set to 5%. The p-value was considered non-significant (NS) at the level of > 0.05, significant at the level of \leq 0.05, 0.01 and highly significant at the level of \leq 0.001. The Pearson linear correlation coefficient, automatic and linear modelling and discriminant analysis were estimated to show the relationship between quantitative parameters Härdle and Simar (2007).

RESULT

Two CMV isolates were obtained from cucumber and squash naturally infected plants showing distinct sever viral like symptoms on cucumber plants while showing mild viral like symptoms on squash plants and conformed by DAS-ELISA using polyclonal antibody specific CMV kits.

The viral infectivity of two CMV isolates were confirmed through sap inoculated mechanically on healthy cucumber *(Cucumis sativus* L. alfa-beta) plants, that gave sever mosaic , blisters and malformation due to **cucumber-CMV** isolate while **squash-CMV** isolate showed mosaic and blisters. As well as gave (+ve) results with CMV polyclonal antibodies by DAS-ELISA.

Photosynthetic pigments content

Photosynthesis is the most vital metabolic processes of plant growth. The effects process in cucumber plant, and infected cucumber plant of two different CMV isolates showed verability responses by assessing the content of chlorophylls and carotenoids in cucumber plants leaves. Graphically data in **Figure (1a, b, c & d)** reduce clarified clearly that, the contents of chlorophylls (a, b and total a+b) and carotenoids were most significant reduction in cucumber plant compared with the healthy ones due to two CMV isolates infection.

Data generated (Fig. 1-c) revealed that contents of total chlorophyll in leaves were significantly decreased in infected cucumber leaves with Gharbia-CMV and Sharqia-CMV isolates by 45% and 31%, respectively.



Fig. 1. Histogram showing the (a) chlorophyll a, (b) chlorophyll b, (c) chlorophyll a+b, and (d) carotenoids level of cucumber. Each value is mean of 6 replicates ± standard error of means, Healthy= Healthy plants, Cucumber-CMV = Infected plants with Cucumber-CMV isolate, Squash-CMV=Infected plants with Squash-CMV isolate.

Biochemical and bioactive contents

The carbohydrates and protein content in cucumber plant healthy and inoculated plant was noticed that difference responses as a result of CMV infection.

Data tableted in **Fig. (2-a)** significantly decreased in infected cucumber plants with Cucumber-CMV and Squash-CMV isolates by 32% and 21%, respectively.

Regarding the effect of two different CMV isolates on total protein content, results in Fig. (2-b) showed significant reduction by 52% and 32% in the total protein contents in cucumber plants leaves that infected with CMV compared to the same healthy one throughout Gharbia-CMV and Sharqia-CMV isolates, respectively. Phenols and proline were estimated in cucumber plants leaves. It was noticed that Cucumber plant showed different responses as a result of two different CMV infections.

Data presented in **Figure (2-d)** shown the effect of two different CMV isolates on total phenol content, results showed significantly increased in the total phenol contents in CMV infected plants compared to the same healthy one at tested cucumber plant, this incident generally throughout two different CMV isolates.

Concerning the impact of two different CMV isolates on total proline content, results in **Figure** (2-c) showed significantly increased in the total proline contents in cucumber plant leaves infected with CMV compared to the same healthy one throughout two CMV isolates.



Fig. 2. Histogram showing the (a) carbohydrates, (b) protein, (c) proline and (d) phenol contents of cucumber plant. Each value is mean of 6 replicates ± standard error of means, Healthy= Healthy plants, Cucumber-CMV = Infected plants with Cucumber-CMV isolate, Squash-CMV=Infected plants with Squash-CMV isolate.

The Gharbia-CMV isolate was more effective than Sharqia-CMV isolate concerning the phenol and proline content of cucumber plant infected that comparing with the same healthy one.

Enzyme activities

Plants employ antioxidants detoxifying enzymes activities to contend oxidative stress generated from biotic stress. The enzymatic activity of PPO, POX, CAT and SOD in cucumber plant under effect of two different CMV isolates was measured to determine the nature of the antioxidant responses of cucumber plant to CMV isolates action.

Cucumber plant indicated different variation in the enzyme activities under the influence of two

different CMV isolates stress. Present work illustrated in generally that, infected cucumber plants with Gharbia-CMV and Sharqia-CMV isolates showed significantly increased in the antioxidant enzyme activity compared to the same healthy one.

Data presented in **Figure (3-a)**, indicated the effect of two CMV isolates on POX activity, results showed significantly increased in the POX activity in Gharbia-CMV and Sharqia-CMV infected plants by 112% and 88%, respectively, compared to the same healthy one.

The high activity of (PPO) was obtained generally, in infected cucumber plants throughout two CMV isolates compared with the same healthy cucumber plants. Data generated in **Figure (3-c)**, showed the changes in the activities of CAT enzyme in cucumber leaves by increased more than four folds and three folds in response to Gharbia-CMV and Sharqia-CMV isolates infection, respectively.

Concerning the effect on SOD activity due to Gharbia-CMV and Sharqia-CMV isolates infected plants, **Figure (3-d)** showed significant increase in the SOD activity by 41% and 29%, respectively, compared to the same healthy one. Among two CMV isolates, the most vital impact on POX, PPO, CAT and SOD activities was Gharbia-CMV isolate.



Fig. 3. Histogram showing the on (a) POX, (b) PPO, (c) CAT, and (d) SOD activities of cucumber plant. Each value is mean of 6 replicates ± standard error of means, Healthy= Healthy plants, Cucumber-CMV = Infected plants with Cucumber-CMV isolate, Squash-CMV=Infected plants with Squash-CMV isolate

DISCUSSION

CMV has a wide distribution in Egypt as detected by (Sofy and Soliman, 2011; Sofy et al 2012).

In current study, both Cucumber-CMV and Squash-CMV isolates were able to induce symptoms when transmitted mechanically through crude

sap to healthy plants but Cucumber-CMV isolate induce sever symptoms while Squash-CMV isolate induce mild symptoms.

At the same time, the two different CMV isolates retorted positively with specific antibodies by applying the DAS-ELISA assay. These results are supported by (Sofy et al 2018).

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Biochemical changes in cucumber plant tissues related to CMV infection as a reduction in the concentration of photosynthetic pigment levels (chlorophyll a, chlorophyll b, total chlorophylls and carotenoids). The current study is in agreement with the study carried by (**Sofy et al 2018**) who told that the decrease in photosynthetic pigments may be attributed to the infected of CMV on biosynthesis of pigments.

Decreased in carbohydrates level in leaves were regarded to be due to CMV isolates infected of cucumber plant as compared to healthy ones; this may be due to the resistance effect of the plant against the infection of virus where the strategy changes from defensive to survival. These results are in agreement with the study carried by (Gupta et al 2010, Montasser et al 2012, and Khalil et al 2014).

Decreased in total protein level in leaves were regarded to be due to CMV isolates infected of tested plant as compared to healthy ones. This observation also was reported for cucumber plants infected with *Cucumber Mosaic virus* (Sofy et al 2018). The total protein decrease was detected also in infected carrot (*Daucus carota* L.) plant (Afreen 2011).

Total phenols act as a significant role in the organizing of plant metabolic process and at all plant growth as well as lignin synthesis (Kumar et al 2010). As well, phenols act as free radical as well as substrates for several antioxidant enzymes (Martin-Tanguy, 2001). Also a number of phenols are considered as pre-infection inhibitors, supplying the plant with a degree of basic resistance against pathogenic micro-organisms. Increased in phenol content level in leaves was regarded to be due to CMV isolates infected of tested plant as compared to healthy ones. This result was agreed with studies reported by (EI-Dougdoug et al 2013; Khalil et al 2014 and Sofy et al 2017). Previous studies suggest that stressed tomato plants accumulate more phenolic compounds as antioxidants to resist viral stress (Hutson and Smith, 1980).

Pathogen infection may effect on the activation of numerous compounds in the cell as the proline production. The obtained results increased in total proline level in leaves and this was regarded to be due to CMV isolates infected of tested plant as compared to healthy ones. These results are agreement with **(Sofy et al 2017)** they reported that stressed tomato plants showed increase in the levels of proline as compared with the nonstressed control plant. Proline accumulation is a common metabolic response to stress; many plants accumulate high amounts of proline in tissues if exposed to any stress (**Pazarlar et al 2013**). Proline accumulation in flax and *faba bean* plants with the rise of salinity levels were in contract with the results obtained by (**Khalil**, **2011**).

Present work concerning the effect of CMV isolates on antioxidant enzymes activity in cucumber plant leaves, showed usually significant increase in the POX, PPO, SOD and CAT activity compared to the healthy ones(non-infected) at all tested plants. These results are in agreement with the study carried by (Huseynova and Aliyev 2012; Sofy et al 2017 and Sofy et al 2018). They observed that activities of leaf antioxidant enzymes have increased with stress. CAT and SOD were over-expressed due to infection of viral, demonstrating their role in detoxification of ROS. POX was reported to be the first enzyme to show changes in its activity under stress (Radwan et al 2007). So was considered POX one of the most important antioxidant enzymes involved in the plant defense response to pathogen attack. The increasing knowledge of plant defense mechanisms against pathogens is casting light on a process that appears to be more and more complex.

CONCLUSION

Both **Cucumber-CMV** and **Squash-CMV** isolates showed different impacts on photopigments, bioactive components and antioxidant enzymes activities in cucumber plants.

The most vital impact was **Cucumber-CMV** isolate.

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التغيرات الأيضيه في نباتات الخيار نتيجة الإصابة بعزلتين مصريتين من فيروس تبرقش الخيار

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> الكلمات الدالة: نبات الخيار، فيروس تبرقش الخيار، إنزيمات الأكسدة، عزلات فيروس تبرقش الخيار، الاليزا

الموجـــــز

يتعرض إنتاج محصول نبات الخيار لخسائر فادحة فى مصر والعالم بسبب الإصابة بفيروس تبرقش الخيار (CMV). لذا فقد ركز البحث على دراسة تأثير اثنين من عزلات CMV المصرية على الأنشطة الأيضية ومضادات الأكسدة فى نبات الخيار. تم حقن نباتات الخيار ميكانيكيا بواسطة العصير الخام المعدى باثنين من العزلات المختلفة لفيروس CMV التى أظهرت اختلافا فى الأعراض الخارجية. تم الكشف عن إصابة نباتات الخيار باثنين من عزلات CMV باستخدام تقنية

DAS-ELISA. تأثرت المركبات البيوكيميائية والنواتج. الأيضية في نبات الخيار المصاب بعزلتي الVMO تسبب في أظهرت الدراسة أن الإصابة بغيروس CMV تسبب في انخفاض كبير في محتوى صبغيات البناء الضوئي والمكونات الكيميائية الحيوية في حين حدثت زيادة في معدلات المكونات النشطة بيولوجيا مثل البرولين والفينول بالإضافة إلى الانزيمات المضادة للأكسدة مثل البيروكسيديز والبولي فينول أوكسيديز والكتاليزوكذلك انزيم سويرأكسيد ديسميوتيز . وذلك بشكل ملحوظ مقارنة مع النباتات غير المصابة. وقد خلصت الدراسة إلى أن عزلة الخيار للمصابة مقارنة بعزلة والأيضية في فيما يتعلق بالدلائل البيوكيميائية والأيضية في نباتات الخيار المصابة مقارنة بعزلة الكوسة نباتات الخيار المصابة مقارنة بعزلة الكوسة

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