The Impact of Hydrogen Peroxide Against Cucumber Green Mottle Mosaic Virus Infection in Watermelon Plants

Deya Eldeen Mohammed Radwan¹, ²*, Khatib Sayeed Ismail²

¹Botany Department, Faculty of Science, Sohag University, Sohag, Egypt
²Virology Section, Biology Department, Faculty of Science, Jazan University, Jazan, Kingdom of Saudi Arabia

Received: 8 November 2019
Accepted: 15 December 2019

Abstract

The aim of this work was to study the effects of hydrogen peroxide (H₂O₂) application against cucumber green mottle mosaic virus (CGMMV) infection in watermelon (Citrullus lanatus) plants. From the obtained results, the induced resistance using H₂O₂ treatments caused a delay in the appearance of CGMMV symptoms in watermelon plants. The viral infection showed abnormal morphological symptoms such as mosaics, yellow blisters and reduction in size. Pretreatment with H₂O₂ before infection was beneficial in increasing the contents of pigments, total proteins, total free amino acids and proline. Consequently, plants appeared morphologically similar to healthy controls. Signaling the effect of the H₂O₂ treatment could induce partial resistance or delay the appearance of symptoms and decreased virus concentration. The induced mechanism of resistance was suggested to be through alterations of plant antioxidant status – both enzymatic and non-enzymatic. All analyzed antioxidant enzymes were induced in response to H₂O₂±CGMMV. Due to the H₂O₂ application prior to infection, malondialdehyde (MDA) content was reduced, indicating a lowering in lipid peroxidation caused by virus infection. On the other hand, internal H₂O₂ and phenolics contents were induced in H₂O₂ + CGMMV-treated leaves. To confirm: total antioxidant activity was increased to be double the value (80.67%) of that recorded in healthy plants (47.18%), indicating changes in antioxidant status as a response to H₂O₂ and/or CGMMV infection. This work provided evidence of the signaling role of exogenous H₂O₂, which led to systemic acquired resistance (SAR) induction acting against CGMMV infection in watermelon plants. From the present findings, a suggestion of spraying of H₂O₂ might be helpful in avoiding the appearance of CGMMV severe symptoms throughout the plants’ life.

Keywords: cucumber green mottle mosaic virus, hydrogen peroxide, systemic acquired resistance, antioxidant enzymes, phenolics, malondialdehyde, antioxidant activity

*e-mail: deya90@yahoo.com
Introduction

Cucumber green mottle mosaic virus (CGMMV) is one of the major cucurbit viral diseases resulting in economic losses in cucurbit production worldwide. CGMMV belongs to the Tobamovirus genus that systemically infects many cucurbitaceous plants such as watermelon (Citrullus lanatus), pumpkin (Cucurbita pepo), squash (Cucurbita moschata), and bottle gourd (Lagenaria siceraria) [1, 2]. CGMMV is spread and transferred easily by many ways, including mechanical transfer, foliage contact, soil contamination, propagation materials, pollen, grains and infected seeds [3-6]. Although there is no insect vector that transmits CGMMV, the widespread nature of the virus is due to the stability of virus particles for a long period under extreme conditions. CGMMV disease causes severe symptoms, including mottling, mosaics, distortion, blistering of leaves and discoloration, and deterioration of the internal part of watermelon fruits – making them undesirable for marketing [1].

Less is known about induced resistance to CGMMV in cucurbit plants, as there are no commercial melon cultivars resistant to CGMMV. Moreover, there are no chemicals used to avoid the spread of CGMMV; only methyl bromide has been used for soil disinfection of CGMMV and has been restricted since 2005. A kind of resistance against CGMMV-SH strain was reported by Crespo et al., [7] in C. sativus accessions through restriction of movement of the virus within the plant. Induction of systemic acquired resistance (SAR) provides immunity against viral infection. SAR induction is a good way to control virus spread. Chemical elicitors of SAR (such as salicylic acid, benzoic acid, amino benzoic acid, oligomers of chitosan, etc.) provide resistance against different virus classes [8]. The mechanism of SAR defense might be through callose deposition and hydrogen peroxide (H$_2$O$_2$) production, which acts as a signal molecule in a plant that activates pathogenesis-related gene expression.

The signaling effect of hydrogen peroxide is well reported as a fast response of plant defense mechanism [9]. Hydrogen peroxide is important for physiological processes of plants. It can control stress responses and systemic signaling [10, 11]. Production of hydrogen peroxide is an early response to environmental stressors due to alterations in biochemical processes [12, 13]. Under stress, hydrogen peroxide is produced in response to oxidative stress. It is a safe form of reactive oxygen species (ROS), where cells can be controlled by an antioxidant enzymatic system. In plant-pathogen interaction, cellular signaling via ROS is generated. Hydrogen peroxide is a non-radical type of reactive oxygen species (ROS). Due to its diffusibility it can move across membranes and reach numerous biomolecules and affect the activity of proteins and act as a signaling factor. H$_2$O$_2$ operates as an oxidant or reductant in many cellular reactions. It can transfer across membranes passively or through water channels [14]. Hydrogen peroxide is less toxic than other ROSs. It is scavenged by H$_2$O$_2$ scavenging enzymes such as catalase, peroxidase and ascorbate peroxidase. As an enzymatic antioxidant, catalase plays a key role in preventing cellular oxidative damage by degrading hydrogen peroxide (H$_2$O$_2$) into water and oxygen with high efficiency [15]. Pan et al. [16] reported exogenous application of H$_2$O$_2$ in Arabidopsis thaliana, which can activate signaling pathways able to produce antioxidants [10]. Recently, it was reported that H$_2$O$_2$ can induce gene expression and enzymatic defense responses in pepper plants [17, 18].

This work was to prove that a protective role of hydrogen peroxide was generated through the induction of SAR in watermelon plants against CGMMV. This role was explained in this article through alterations of both enzymatic and non-enzymatic antioxidant status of plants.

Materials and Methods

Plant Material and Treatments

A six-week experiment was carried out to test the effects of hydrogen peroxide (Sigma Aldrich, USA. 50 wt. % in H$_2$O) in induction of resistance against CGMMV-SH infection in watermelon plants. Seeds of Citrullus lanatus (cv. Malali) were germinated in soil (sand and clay 3:1) in 3 L pots in a greenhouse. Completely randomized design was used in this study. Three weeks after planting, plants were grouped into 6 groups (each group consists of 4 pots containing 3-4 plants, sampling from 4 independent plants). Groups were treated according to the following:

1. Control: Plants were sprayed with tap water.
2. Infected: Plants were treated with tap water one day prior CGMMV inoculation.
3. 10 mM H$_2$O$_2$: Plants were sprayed with 10 mM solution of H$_2$O$_2$.
4. 5 mM H$_2$O$_2$: Plants were sprayed with 5 mM solution of H$_2$O$_2$ one day prior inoculation.
5. 10 mM H$_2$O$_2$: Plants were sprayed with 10 mM solution of H$_2$O$_2$ one day prior inoculation.
6. 20 mM H$_2$O$_2$: Plants were sprayed with 20 mM solution of H$_2$O$_2$ one day prior inoculation.

For inoculation, freshly infected leaves found to be positive for CGMMV were ground in a mortar containing 0.1 M phosphate buffer of pH = 7.0 (1:2 w/v). The homogenate was then filtered through two layers of muslin. Plants were dusted with 600-mesh carborundum and rubbed gently with a cotton swap previously dipped into the suspension of virus inoculum [1]. Plants were inoculated once throughout the experimental period. Control plants were treated with carborundum and phosphate buffer only. Natural infections were avoided by keeping plants in isolated controlled conditions (growth chamber). Plants were
kept at 20±2°C and 65% humidity in a growth chamber. Symptoms appeared two weeks after inoculation. Leaf samples were photographed and taken for analyses after three weeks of virus inoculation (i.e., at the end of this experiment).

DAS-ELISA for CGMMV Detection

100 Healthy and infected plants were subjected to a DAS-ELISA test (double antibody sandwiched-enzyme linked immunosorbent assay) [19]. A ready for use kits (Agdia Inc., Madison, USA) were used to analyze the samples. All the buffers, coating antibodies and conjugated antibodies were diluted as the protocol provided by the manufacturer. Absorbance at 405 nm was determined with a plate reader for all samples at the same time.

Percentage of Infection and Severity of Symptoms

Three weeks after inoculation the percentage of infected plants and the severity of symptoms were examined using the following rating scale: 0 = no symptoms; 1 = chlorotic local lesions and mild mosaic; 2 = severe mosaic and 3 = blisters and malformation. Disease severity values were calculated using the following equation [20]:

\[
\text{Disease severity} (DS) = \frac{\sum (\text{disease grade} \times \text{number of plants in each grade})}{(\text{Total number of plants} \times \text{highest disease grade})}
\]

Three weeks after inoculation the youngest fully developed leaves from both control and treated plants were sampled for biochemical analyses.

Photosynthetic Pigments Content

The analysis of Chlorophyll A (Chl A), Chlorophyll B (Chl B) and Carotenoids (Cars) was carried out using the Lichtenthaler and Buschmann Method [21]. Fresh leaves (0.1 g) of control and treated fresh leaves were extracted by grinding in 10 ml acetone (85%) in a mortar. The extract was centrifuged at 5000 rpm. Supernatant was separated in a test tube for analysis. Pigment extract was then analyzed by colorimeter (T80 UV-VIS spectrophotometer, PG Instruments, UK) using three wavelengths: 663, 647 and 470 nm.

The absorbance of the extracts was then used to calculate pigment concentrations through the following formulas:

\[
\text{Chlorophyll a} = 12.25 A_{663} - 2.79 A_{647}
\]
\[
\text{Chlorophyll b} = 21.50 A_{647} - 5.10 A_{663}
\]
\[
\text{Carotenoids} = (1000 A_{470} - 1.82*\text{Chl a} - 95.151*\text{Chl b})/225
\]

The contents of pigments were calculated as mg g⁻¹ FW.

Antioxidant Enzyme Analyses

Fresh leaves (0.5 g) of control and treated plants were extracted in 10 ml of phosphate buffer (pH = 7.0) by grinding in a mortar. The extract was then centrifuged at 14000 rpm in cool conditions 4°C for 20 min. The supernatant was separated and prepared for analysis of antioxidant enzymes (peroxidase; POD, catalase; CAT, ascorbate peroxidase; APX and superoxide dismutase; SOD).

For analysis of POD activity we used the Zhang [22] method. The extract was added to the analyzing medium containing 5 mM guaiacol, 0.3 mM hydrogen peroxide, and 0.1 mM EDTA in 40 mM phosphate buffer (pH = 7.2). The increase in oxidation of guaiacol was analyzed at 470nm using a UV-VIS spectrophotometer (T80, PG Instruments, UK). Using the extinction factor = 26.2 mM cm⁻¹, the POD activity was calculated as μmol of guaiacol oxidized min⁻¹ g⁻¹ FW.

In the case of CAT activity we used the Chandlee and Scandalios method [23]. The enzyme extract was mixed with the assay medium that contained 10mM H₂O₂ in 25 mM potassium phosphate buffer (pH 7.0). The rate of decomposition of H₂O₂ was then detected at 240 nm, expressing CAT activity as μmol min⁻¹ H₂O₂ (Extinction factor = 0.036mM cm⁻¹).

For APX activity analysis we used the method of Nakano and Asada [24]. The assay medium consisted of 0.3 mM ascorbate, 0.1 mM EDTA, and 0.06 mM H₂O₂ in 50 mM phosphate buffer (pH 7.0). The analysis was based on the decrease of ascorbate by determination of the decline in absorbance at 290 nm (extinction factor = 2.8 mM cm⁻¹).

SOD activity was determined using the Beauchamp and Fridovich [25] method. The extract was added to the assay medium, which contained 9.9 mM L-methionine, 0.025% (w/v) nitroblue tetrazolium (NBT), and 0.0044% (w/v) riboflavin in 50 mM phosphate buffer (pH 7.8). Photo-reduction of NBT (purple color) was analyzed at 560nm. One unit of SOD enzyme extract caused 50% inhibition of photo-reduction of NBT.

Antioxidant Metabolites MDA and H₂O₂

Malondialdehyde (MDA) content was determined in leaves by the Zhang method [22]. Fresh leaves were extracted in 5% TCA (trichloroacetic acid), followed by 10 min centrifugation at 3000 rpm. The supernatant was then mixed with 0.03 mM of 2-thiobarbituric acid (TBA) and incubated at 94°C for 15 min. After cooling, the developed color was then analyzed by spectrophotometer at 532 nm. Using extinction factor (E = 155 mM cm⁻¹), the MDA concentration was calculated as nmol MDA g⁻¹ FW.

Hydrogen peroxide (H₂O₂) content was determined by using the method of Jana and Choudhuri [26]. Leaves were extracted in 50 mM phosphate buffer (pH = 6.5) and centrifuged at 6000g for 25 min. The supernatant
was mixed with 1 ml of 0.1% titanium sulfate in
20% H₂SO₄ after the ppt formation; the mixture was
centrifuged at 6000g for 15 min. The pellet was then
dissolved in 5 ml H₂SO₄ (2M) and the absorbance was
then measured by spectrophotometer at 410 nm. Using
the extinction coefficient (E = 0.28 µmol⁻¹ cm⁻¹), H₂O₂
content was calculated as nmol g⁻¹ FW.

Phenolics Content

A folin- Ciocalteau reagent was used to determine
the content of phenolics in control, infected and
treated leaves. The method of Singleton and Rossi [27]
was used. Extracts were prepared in methanol (80%)
and mixed with 1.8 ml of Folin-Ciocalteu reagent
diluted 1:10 and stand for 5 min at room temperature.
1.2 ml of NaHCO₃ (7.5% w/v) was added. Color was
analyzed at 765 nm after standing for 60 min at room
temperature. Phenolic contents were presented as
µg GAEG⁻¹ DW, where GAE is the equivalent of gallic
acid.

Assay of Total Antioxidant Activity

DPPH (1,1-diphenyl-2-picrylhydrazyl, Sigma
Aldrich) free radical scavenging assay test was used
to analyze the antioxidant status of control and treated
leaves [28]. Fresh leaves were extracted in methanol
and mixed with a similar amount of freshly prepared
DPPH solution (80 ppm in methanol). After mixing
thoroughly, tubes were kept in the dark for 30 min.
The color was then determined by spectrophotometer at
517 nm. Using the following equation, total antioxidant
activity was calculated as a percentage of DPPH
scavenging activity:

Antioxidant activity = [1-(Aᵢ⁻Aₐ)/Aᵢ]*100

where Aᵢ is absorbance of extract+ DPPH, Aᵢ is the
absorbance of extract + methanol and Aᵢ is absorbance
of DPPH + methanol.

Proteins Content

Total proteins content of control, infected and
H₂O₂-treated leaves were analyzed using the Lowry
method [29]. Samples were extracted in NaOH (0.1N)
in water bath (100°C) for 1 hr. The extracts were
centrifuged at 4000 rpm and the supernatant was
taken for analysis. Alkaline-Folin reagent was used.
One ml of extract was mixed with 5 ml of alkaline
reagent prepared as follows (reagent A: 2% Na₂CO₃ in
0.1N NaOH and reagent B: 0.5% CuSO₄ in Sod. Pot.
Tartrate). After standing 20 min the folin reagent 0.5 ml
was added and mixed thoroughly and left to stand for
20 min. Absorption was determined at 750 nm. Total
protein contents were expressed as mg /g dry weight of
leaves.

Total Free Amino Acids

The Moore and Stein [30] method was used to
analyze the free amino acids of different leaves. Tissue samples were extracted in distilled water by
heating in a water bath at 90°C for 2 hrs. The extracts
were then centrifuged and the supernatants were
collected. Supernatant was added to 1 ml of ninhydrin
solution with stannous chloride. The mix was heated
in a boiling water bath for 20 min; a purple color
developed. Diluents (5 ml) were added and contents
were mixed. Fifteen minutes later, the developed color
was read at 570 nm against blank. The free amino acid
concentrations were calculated as mg/g dry matter.

Proline Content

The method of Bates et al. [31] was used to
determine the proline content control, infected and
H₂O₂-treated leaves. The extract was prepared using
0.1 g of dried powdered leaves in 10 ml of 3%
sulfsaliclyc acid for 12h. Centrifuging was carried
out for 10 min at 1500 rpm. For analysis, supernatants
were mixed with acid ninhydrin reagent +2 ml glacial
acetic acid and heated in a water bath at 100°C for
1 h. Cooling of the mixture using ice bath then 4 ml
toluene were added for extraction of the pink color. The
absorbance was measured at 520 nm for toluene phase
containing the color. Proline content was calculated as
µg g⁻¹ DW using a pre-analyzed standard curve using
proline amino acid.

Statistical Analysis

The results were reported as mean±SD of four
independent replicates. Statistical analyses of data were
carried out by computer using SPSS ver. 23.0 software.
One-way ANOVA and least significant differences test
(LSD) for multiple comparisons were used to evaluate
the differences among the means.

Results

Morphological Changes and Growth Analysis

Fifteen days after inoculation, leaves became mosaic
with green and yellow blisters and were reduced in size
compared to the control (Fig. 1). The leaf was mostly
yellow with heavy hairy surface — especially in the
petiole and leaf edges. Infected leaf area was reduced
to 79% less than control. Treatment with H₂O₂ without
infection increased leaf area to 12% more than control.
The application of 20 mM H₂O₂ in water bath at 90°C
for 2 hrs. The extracts were then centrifuged and the supernatants were
collected. Supernatant was added to 1 ml of ninhydrin
solution with stannous chloride. The mix was heated
in a boiling water bath for 20 min; a purple color
developed. Diluents (5 ml) were added and contents
were mixed. Fifteen minutes later, the developed color
was read at 570 nm against blank. The free amino acid
concentrations were calculated as mg/g dry matter.

Proline Content

The method of Bates et al. [31] was used to
determine the proline content control, infected and
H₂O₂-treated leaves. The extract was prepared using
0.1 g of dried powdered leaves in 10 ml of 3%
sulfsaliclyc acid for 12h. Centrifuging was carried
out for 10 min at 1500 rpm. For analysis, supernatants
were mixed with acid ninhydrin reagent +2 ml glacial
acetic acid and heated in a water bath at 100°C for
1 h. Cooling of the mixture using ice bath then 4 ml
toluene were added for extraction of the pink color. The
absorbance was measured at 520 nm for toluene phase
containing the color. Proline content was calculated as
µg g⁻¹ DW using a pre-analyzed standard curve using
proline amino acid.

Statistical Analysis

The results were reported as mean±SD of four
independent replicates. Statistical analyses of data were
carried out by computer using SPSS ver. 23.0 software.
One-way ANOVA and least significant differences test
(LSD) for multiple comparisons were used to evaluate
the differences among the means.
followed by GCMMV infection. A decrease in leaf area and shoot length of the infected plants led to an obvious decrease in dry matter. Lowered dry matter of watermelon plants by 49% was recorded with infection. In contrast, a gradual increase in dry matter was noticed with H\textsubscript{2}O\textsubscript{2} treatment followed by infection. Spraying 20 mM H\textsubscript{2}O\textsubscript{2} before infection was able to increase dry matter by up to 60% more than control (Table 1).

Changes in water content of infected and H\textsubscript{2}O\textsubscript{2}-treated plants was observed. A highly significant decrease of water content in both infected and (5 mM H\textsubscript{2}O\textsubscript{2} + inf) plants was noticed, while 20 mM H\textsubscript{2}O\textsubscript{2} followed by infection was almost similar to the control (Table 1).

Both percentage of infection and disease severity showed a noticeable decrease with H\textsubscript{2}O\textsubscript{2} pretreatment in inoculated plants. Although all concentrations of H\textsubscript{2}O\textsubscript{2} (5-20) were able to decrease the infection percentage, the most effective concentration was the highest one (20mM H\textsubscript{2}O\textsubscript{2}). This concentration was able to minimize the percentage of infection and lowered the severity of disease (Table 2). The concentration of CGMMV virus using the ELISA test showed positive results in infected and (H\textsubscript{2}O\textsubscript{2} + virus) treated leaves. This means the appearance of symptoms in plants with different degrees according to the detected concentration of the virus. It was noticed that the concentration declined gradually by increasing the applied H\textsubscript{2}O\textsubscript{2} dose (Table 2).

Photosynthetic Pigments Content

The analyzed photosynthetic pigment contents in infected leaves showed a highly significant reduction in Chl A, Chl B and Carotenoids (Fig. 2). This reduction reached 50% of control in Chl A and carotenoids due to virus infection. The use of H\textsubscript{2}O\textsubscript{2} without a virus showed a highly significant increase of photosynthetic pigment contents (55, 39 and 25% more than control in case of Chl A, Chl B and cars, respectively). On the other hand, H\textsubscript{2}O\textsubscript{2} treatments followed by virus infection could pronouncedly increase the contents of photosynthetic pigments. It was noticeable that the increase in pigments was concomitant with H\textsubscript{2}O\textsubscript{2} concentrations – especially in Chl A and Chl B. It is noticeable that 10 and 20 mM H\textsubscript{2}O\textsubscript{2} + infections were more effective in pigment content improvement. The most obvious result was obtained with the treatment 20 mM H\textsubscript{2}O\textsubscript{2} + virus infection, which led to increasing Chl A, Chl B and carotenoids to be 69, 94 and 42% more than their corresponding controls (Fig. 2).

Table 1. Effect of CGMMV infection and H\textsubscript{2}O\textsubscript{2} treatments on Leaf area (cm\textsuperscript{2} plant\textsuperscript{-1}), Shoot length (cm plant\textsuperscript{-1}), Water content (%) and Dry matter (g plant\textsuperscript{-1}) of watermelon plants. The values are means (M) of four replicates±standard deviation (SD). The values are means (M) of four replicates±standard deviation (SD).

| Treatments | Leaf area (cm\textsuperscript{2} plant\textsuperscript{-1}) | Shoot length (cm plant\textsuperscript{-1}) | Water content (%) | Dry matter (g plant\textsuperscript{-1}) |
|------------|----------------------------------------------------------|----------------------------------------------|-------------------|----------------------------------------|
|            | M±SD | %       | M±SD | %       | M±SD | %       | M±SD | %       |
| Control    | 105.92±9.87 | 100 | 43.64±5.38 | 100 | 87.34±0.49 | 100 | 3.73±0.09 | 100 |
| Infected   | 22.15**±12.67 | 20.91 | 19.87**±4.39 | 45.53 | 76.48**±1.94 | 51.74 | 1.93**±0.04 | 93.17 |
| 10 mM H\textsubscript{2}O\textsubscript{2} | 111.37±11.65 | 105.15 | 45.65±6.34 | 104.61 | 89.95±0.72 | 106.97 | 3.99±0.02 | 110.97 |
| 5 mM H\textsubscript{2}O\textsubscript{2} + inf | 78.29**±8.23 | 73.91 | 62.59**±2.85 | 143.42 | 77.13**±0.81 | 115.82 | 4.32±0.06 | 119.32 |
| 10 mM H\textsubscript{2}O\textsubscript{2} + inf | 96.17**±15.74 | 90.79 | 69.13**±3.46 | 158.41 | 83.85**±0.95 | 153.35 | 5.72**±0.03 | 156.35 |
| 20 mM H\textsubscript{2}O\textsubscript{2} + inf | 100.33±10.63 | 94.72 | 77.62**±4.64 | 177.86 | 87.54±0.37 | 160.32 | 5.98**±0.05 | 164.32 |

Statistical significance of differences compared to control: *, significant at $P<0.05$; **, significant at $P<0.01$.
Antioxidant Enzyme Activities POD and CAT

Activities of POD and CAT were induced significantly in the infected leaf samples (Table 3). The activities were almost doubled in response to infection. With all H$_2$O$_2$ treatments, POD and CAT showed enhanced activities compared to control. Leaves treated with 10 mM H$_2$O$_2$ induced POD and CAT activities to be 86% and 80% more than those of control, respectively. A highly significant increase in POD and CAT activities was noticed with the increase of H$_2$O$_2$ treatment followed by infection. This increase reached 182% (POD) and 118% (CAT) more than control in the case of spraying 20 mM H$_2$O$_2$ prior to infection.

APX and SOD Activities

It was noticeable that APX activity in infected leaves was highly induced (Table 3). Compared to control, APX activity was 3-fold higher (220% increase) in response to GCMMV infection. SOD activity increased by 25% more than control due to virus infection. Inductions of APX and SOD activities were recorded in leaves treated with 10mM H$_2$O$_2$ without infection. The application of H$_2$O$_2$ (5-20 mM) followed by infection caused a concomitant decrease in APX activity. On the
The Impact of Hydrogen Peroxide... 3777

other hand, leaves treated with H2O2 only showed higher SOD activities compared to the control. Moreover, an increase of (141%) more than control was recorded in SOD activity in watermelon leaves sprayed with 20 mM H2O2 plus infection.

Antioxidant Metabolites MDA and H2O2

The amounts of both MDA and H2O2 were analyzed in control and treated leaves (Table 4). MDA was commonly used as an indicator for lipid peroxidation and oxidative stress. An increase in MDA content was noticed with infection (about 37% more than control). The use of H2O2 prior to infection showed variable results. H2O2 without infection was able to lower the MDA content by 5%, but with infection MDA contents increased even if pretreated with H2O2. On the other side, H2O2 content increased as a response to infection (65% more). Pretreatments with H2O2+infection were able to accumulate H2O2 and the amounts were dose-dependent. The highest amount of H2O2 was recorded in leaves treated with 20 mM H2O2 followed by infection. Compared with the amounts present in infected leaves, H2O2 couldn’t record the same ratio of increase with the applied H2O2 concentrations.

Total Phenolics and Antioxidant Activity

Analysis of phenolics content in control and CGMMV-infected leaves revealed the accumulation of amounts of phenolics in infected leaves by 45% (Table 4). Treatment with 20 mM H2O2 without virus infection was able to increase phenolics slightly (9%), while 20 mM H2O2 plus infection increased phenolics content by 23% more than control. With all concentrations of H2O2 followed by infection, the phenolics content recorded a highly significant increase that reached 22-30% more than that of control. The antioxidant activity of the tested leaves showed significant differences. In general, all treatments could increase the antioxidant activity of leaves. Infection with CGMMV induced AOA to be 66% while control leaves recorded AOA of about 47%. In the case of H2O2 treatments, the increase in AOA was concentration dependent. Moreover, the highest improvement of AOA was noticed in leaves treated with 20 mM H2O2+infection, which reached 81-85%.

Table 3. Effect of CGMMV infection and H2O2 treatments on Peroxidase, Catalase, Ascorbate Peroxidase and Superoxide dismutase activities (Unit g-1FW) of watermelon plants. The values are means (M) of four replicates±standard deviation (SD). The values are means (M) of four replicates±standard deviation (SD).

| Treatments | POD (Unit g-1FW) | CAT (Unit g-1FW) | APX (Unit g-1FW) | SOD (Unit g-1FW) |
|------------|-----------------|-----------------|-----------------|-----------------|
|            | M±SD            | %               | M±SD            | %               |
| Control    | 94.83±3.27      | 100             | 43.64±2.94      | 100             |
| Infected   | 187.07**±6.34   | 197.27          | 88.41**±0.57    | 202.59          |
| 10 mM H2O2 | 176.42**±4.83   | 186.04          | 78.60**±0.22    | 180.11          |
| 5 mM H2O2 + inf | 234.53**±3.62 | 247.32          | 86.37**±0.84    | 197.91          |
| 10 mM H2O2 + inf | 274.36**±8.75 | 289.32          | 93.76**±0.74    | 214.85          |
| 20 mM H2O2 + inf | 266.95**±5.83 | 281.50          | 95.04**±1.53    | 217.78          |

Statistical significance of differences compared to control: *, significant at P<0.05; **, significant at P<0.01.

Table 4. Effect of CGMMV infection and H2O2 treatments on MDA (μmol MDA g-1FW), cellular H2O2 (nmol H2O2 g-1FW), phenolics (μg g-1FW) and antioxidant activity (%) of watermelon plants. The values are means (M) of four replicates±standard deviation (SD).

| Treatments | MDA (μmol MDA g-1FW) | H2O2 (nmol H2O2 g-1FW) | Total Phenolics (μg g-1FW) | AOA (%) |
|------------|----------------------|------------------------|---------------------------|---------|
|            | M±SD                 | %                      | M±SD                      | %       |
| Control    | 15.64±0.93           | 100.00                 | 19.72±0.38                | 100.00  |
| Infected   | 26.37**±1.43         | 136.64                 | 32.54**±1.56              | 165.01  |
| 10 mM H2O2 | 14.82±0.56           | 94.76                  | 21.32±0.52                | 108.11  |
| 5 mM H2O2 + inf | 21.34*±0.71 | 136.45                 | 23.96*±0.45               | 121.50  |
| 10 mM H2O2 + inf | 21.95*±0.65 | 140.35                 | 25.63*±0.71               | 129.97  |
| 20 mM H2O2 + inf | 19.84*±1.06 | 126.85                 | 26.45*±0.87               | 134.13  |

Statistical significance of differences compared to control: *, significant at P<0.05; **, significant at P<0.01.
Table 5. Effect of CGMMV infection and H₂O₂ treatments on Total proteins (mg g⁻¹FW), Total free amino acids (mg g⁻¹DW) and Proline (mg g⁻¹DW) contents of watermelon plants. The values are means (M) of four replicates±standard deviation (SD).

| Treatments  | Total proteins (mg g⁻¹DW) M±SD | % | Total free amino acids (mg g⁻¹DW) M±SD | % | Proline content (mg g⁻¹DW) M±SD | % |
|-------------|--------------------------------|---|----------------------------------------|---|---------------------------------|---|
| Control     | 218.78±12.54                  | 100.0 | 38.17±0.30                             | 100.0 | 1.18±0.01                       | 100.0 |
| Infected    | 276.81±11.65                  | 126.52 | 55.95±0.64                             | 146.58 | 3.27±0.03                       | 277.12 |
| 10 mM H₂O₂ | 332.16±17.83                  | 152.82 | 47.29±0.48                             | 123.89 | 2.48±0.02                       | 210.17 |
| 5 mM H₂O₂ + Inf | 302.53±32.45               | 138.28 | 48.37±0.25                             | 126.72 | 2.76±0.04                       | 233.90 |
| 10 mM H₂O₂ + Inf | 354.34±18.38              | 161.96 | 56.68±0.63                             | 148.49 | 2.68±0.03                       | 227.12 |
| 20 mM H₂O₂ + Inf | 365.45±19.59              | 167.04 | 60.86±0.55                             | 159.44 | 2.37±0.04                       | 200.85 |

Statistical significance of differences compared to control: *, significant at P<0.05; **, significant at P<0.01.

Total Proteins, Total Free Amino Acids and Proline Contents

The amounts of total proteins are presented in Table 5. Infected leaves showed significant accumulation of proteins due to infection. In infected leaves, the increase in proteins reached 27% more than control. H₂O₂ treatment prior to infection was able to raise the protein content. In detail, the application of 5 mM H₂O₂ + infection caused a 38% increase and 20 mM H₂O₂ + infection caused a 67% increase compared to control. Similar to proteins, free amino acid contents followed the same behavior of increase in treated samples. The free amino acid of infected leaves reached 47% more than the control. All H₂O₂ treatments increased the proline content to 101% compared to that of control.

Discussion

Induction of systemic acquired resistance (SAR) in plants is an efficient way to resist pathogen infection. SAR occurs in many plant species in response to pathogen infection and the application of certain chemicals. SAR is associated with the expression of plant defense genes [32]. Chemical elicitors can generate systemic signals that lead to SAR induction. Hydrogen peroxide is one of the chemical inducers of SAR, which in turn signals the formation of pathogenesis-related proteins, and phytoalexins [33]. Hydrogen peroxide and superoxide act directly as second messengers in the regulation and expression of the genes encoding proteins responsible for oxidative stress response [13]. Hydrogen peroxide is a versatile molecule that acts as a non-radical reactive oxygen species (ROS). Harmful and beneficial consequences of ROS have been recorded [34, 35]. Hydrogen peroxide is safe and can be easily detoxified by an antioxidant enzyme system; catalase, peroxidase, ascorbate peroxidases. It is well reported that H₂O₂ plays a signaling role in plants under stress and pathogen defense [36-38] – especially against virus infection, as reported by Mejía-Teniente and Durán-Flores et al. [18]. In this work, treatment with H₂O₂ improves the growth of watermelon plants and protects against CGMMV infection. This improvement was noticed in increased growth, shoot lengths, dry matter, leaf area, minimized percentage of infection and lowered severity of disease and lowered virus concentrations. This is the first report about induced resistance using H₂O₂ treatment against CGMMV in watermelon plants.

Due to leaf CGMMV symptoms and mosaics in infected samples, the photosynthetic pigments content was significantly altered compared with control. Treatment with H₂O₂+infection was able to increase amounts of chl a, chl b and carotenoids of leaves significantly. The increase was concomitant with the concentration of H₂O₂ [36], treatments with which increased PSII photochemical efficiency. Moreover, H₂O₂ treatments increased the activity of Rubisco. Increased content of pigments reflects more efficiency of photosynthesis process in plants treated with H₂O₂. In support, Ashfaq et al. [39] reported the promotion of photosynthesis in wheat plants after exogenous application of 100 nM H₂O₂. Moreover, treatment with H₂O₂ exogenously to quinoa plants was able to improve the photosynthesis rate by 42% and increase chlorophyll content by 36% more than the control.

In this work, a resistance mechanism against CGMMV using an exogenous application of H₂O₂
in watermelon plants was suggested. This induced resistance occurred through alterations of an antioxidant (enzymatic and non-enzymatic) system due to the signaling effect of H$_2$O$_2$. A significant increase of activity of POD, CAT and APX in response to 10mM H$_2$O$_2$ without infection was observed. The increase reached 86%, 80% and 77% more than control for POD, CAT and APX, respectively. On the other hand, the increase in POD, CAT, and APX with infection was 182%, 118%, and 70% more than control. These are H$_2$O$_2$ scavenging enzymes, and increasing their activities indicates the formation or presence of more amounts of H$_2$O$_2$ [40, 41]. More increase in POD and CAT activities with H$_2$O$_2$ treatment and CGMMV was because of the double source of induction; the pathogen and exogenous H$_2$O$_2$ application. Moreover, SOD activity was induced by infection, and H$_2$O$_2$ was able to induce SOD with or without the presence of infection. SOD is induced under biotic and abiotic stress and functions in dismutation of O$_2^-$ to produce H$_2$O$_2$ [42]. In this work, a 26% increase in SOD activity with infection might be to avoid oxidative stress produced by infection. On the other hand, spraying H$_2$O$_2$ caused highly significant induction of SOD (141% more than control). This is in accordance with the induced activity of H$_2$O$_2$ scavenging enzymes (POD, CAT and APX).

In support are previous studies done by Clarke et al., [43] who reported changes in antioxidant activities as a response to WCIMV in Phaseolus vulgaris leaves. Moreover, changes in antioxidant enzymes were detected in biotic stress (ZYMV infection to pumpkin plants) [44, 45] and abiotic stress (herbicides application to peanut plants) by Radwan et al. [46]. Internal production of H$_2$O$_2$ or receiving exogenous help increased the signaling effect of H$_2$O$_2$ and induction of SAR against virus infection. Consequently, antioxidant enzymes changes, an increase in H$_2$O$_2$ levels or accumulation was noticed. This accumulation of H$_2$O$_2$ with infection and other treatments indicates oxidative stress as a response to CGMMV and H$_2$O$_2$ signaling cascade. As a defense to pathogen infection, radical and non-radical ROS are accumulated, triggering the oxidative burst that leads to recognition of the pathogen. H$_2$O$_2$ serves as a defensive barrier against pathogen infection [47].

MDA is a final product of fatty acid peroxidation caused by ROS accumulation [48]. MDA is used as an indicator for lipid peroxidation and oxidative stress due to the overproduction of ROS because of virus infection [49, 50]. In the present, CGMMV infection caused the accumulation of MDA, indicating lipid peroxidation and oxidative stress. The MDA amount was lowered by exogenous spraying of H$_2$O$_2$ to the plants prior to infection. The signaling effect of H$_2$O$_2$ induces some preparatory defense mechanism prior to infection. This defense might be through the formation of special membrane proteins, preventing cell membrane peroxidation or lowering MDA amount. These proteins were induced in advance to be used further against infection. In the present study, analysis of total proteins showed the accumulation of more proteins as a response to infection and H$_2$O$_2$ spraying, i.e., protective action against infection was obtained. They are pathogenesis-related proteins or SAR proteins. The higher the amount of H$_2$O$_2$ level, the higher the proteins accumulated. 20mM H$_2$O$_2$ application followed by infection caused a 67% increase in proteins than control because of the double source of induction: treatment and infection. Proteins are among the main types of anti-plant viral substances [51]. Plants form certain proteins upon infection (pathogenesis-related proteins) to restrict pathogen growth and multiplication [52]. Pathogenesis-related proteins are molecular markers of SAR responses [53] that can be induced both by infection through activation of PR genes and by the exogenous application of chemical elicitors. They are plant defenses in the form of SAR, which is a broad level of resistance [8]. In this work, increasing free amino acids and proline contents upon infection and/or H$_2$O$_2$ application was noticed. Li et al. [54] reported that the accumulation of amino acids is among the pathways by which different genes are responsible for interactions between CGMMV and watermelon. For most compounds, sulfur and nitrogen are mainly synthesized from amino acids [55]. In this experiment, proline amino acid analysis revealed accumulation in response to CGMMV and H$_2$O$_2$ treatments. Proline amino acid is considered an antioxidant and ROS scavenger and functions in maintaining membrane integrity and stabilizer of both antioxidant enzymes and subcellular structures [56]. It is used in activating detoxification mechanisms, and functions in the signaling process. As previously reported, proline protects against H$_2$O$_2$-induced oxidative stress of wild almond plants by increasing antioxidant enzyme activities and by decreasing MDA content and membrane damage [57].

Phenolics are among the secondary metabolites that are involved in plant protection [58]. In this study, the accumulation of phenolics content was noticed, accompanied with infection and/or H$_2$O$_2$ treatments. This is in accordance with the results obtained by Li and An et al. [54], who reported significant increases in phenolic compounds due to CGMMV infection. Phenolic compounds were previously reported to have antioxidantizing activity providing a self-defense role under stressful conditions [59, 60]. Phenolics have the ability to alter peroxidation properties by lowering the peroxidative reaction of membranes [61]. In this experiment, the increase in phenolics related to H$_2$O$_2$ treatments can be explained as their capability of being either proton or electron donors, hence they can participate in scavenging free radicals [62]. Scavenging oxygen radicals depends on the ability of the antioxidant system of the plant. This ability to scavenge oxygen radicals is known to be inversely proportional with the levels of lipid peroxidation [63]. To confirm the effect of H$_2$O$_2$ treatment on the antioxidant status and its protective action, the total antioxidant activity was
analyzed. It was found that the AOA was doubled to be 85% with 20 mM H$_2$O$_2$ + infection while the control records AOA of 47%. This increase in antioxidant activity might be due to the signaling effect of H$_2$O$_2$ sprayed prior to infection, which could induce SAR through the accumulation of proteins, phenolics, proline, and free amino acids contents and alteration of antioxidant enzyme activities.

Conclusions

This work investigated the impacts of H$_2$O$_2$ treatment against CGMMV infection in watermelon plants. The treatment delayed infection and the appearance of severe CGMMV symptoms in watermelon plants. Plants treated with H$_2$O$_2$+ CGMMV showed normal appearance as healthy plants during the experimental period. This can be discussed through alteration of both the enzymatic and non-enzymatic antioxidant status due to H$_2$O$_2$ treatment prior to infection. Moreover, H$_2$O$_2$ treatments lowered MDA content but accumulated proteins, free amino acids, cellular H$_2$O$_2$, proline and phenolics contents. These alterations in antioxidant status and contents of biochemical constituents due to H$_2$O$_2$ treatments can suggest a certain role of H$_2$O$_2$ in delay or resisting CGMMV infection in watermelon plants.

Conflict of Interest

The authors declare no conflict of interest.

References

1. REINGOLD V., LACHMAN O., KOREN A., DOMBROVSKY A. First report of Cucumber green mottle mosaic virus (CGMMV) symptoms in watermelon used for the discrimination of non-marketable fruits in Israeli commercial fields. New Dis. Rep. 28(11), 2013.
2. PITMAN T., POSIS K., TIAN T., BELANGER C., ROY A., FALK B. First Report of Watermelon Green Mottle Mosaic Virus in North America. Plant Disease. PDIS, 2019.
3. LI J. X., LIU S. S., GU Q. S. Transmission efficiency of Cucumber green mottle mosaic virus via seeds, soil, pruning and irrigation water. J. Phytopathol. 164(5), 300, 2016.
4. LIU H., LUO L., LI J., LIU P., CHEN X., HAO J. Pollen and seed transmission of Cucumber green mottle mosaic virus in cucumber. Plant Pathol. 63(1), 72, 2014.
5. REINGOLD V., LACHMAN O., BLAOSOV E., DOMBROVSKY A. Seed disinfection treatments do not sufficiently inhibit the infectivity of Cucumber green mottle mosaic virus (CGMMV) on cucurbits seeds. Plant Pathol. 64(2), 245, 2015.
6. WU H., QIN B., CHEN H., PENG B., CAI J., GU Q. The rate of seed contamination and transmission of Cucumber green mottle mosaic virus in watermelon and melon. Sci Agric Sin. 44(7), 1527, 2011.
7. CRESPO O., JANSSEN D., ROBLES C., RUIZ L. Resistance to Cucumber green mottle mosaic virus in Cucumis sativus. Euphytica. 214(11), 201, 2018.
8. TRIPATHI D., RAJKHY G., KUMAR D. Chemical Elicitors of Systemic Acquired Resistance-Salicylic Acid and Its Functional Analogs. Current Plant Biology. 2019.
9. HABIBI G. Hydrogen peroxide (H$_2$O$_2$) generation, scavenging and signaling in plants. Oxidative Damage to Plants: Elsevierpp. 557, 2014.
10. AHMAD P. Oxidative damage to plants: antioxidant networks and signaling: Academic Press; 2014.
11. HOSSAIN M.A., BHATTACHARJEE S., ARMIN S.-M., et al. Hydrogen peroxide priming modulates abiotic oxidative stress tolerance: insights from ROS detoxification and scavenging. Frontiers in plant science. 6, 420, 2015.
12. BEN REJEB K., LEFEBVRE-DE VOS D., LE DISQUET I., et al. Hydrogen peroxide produced by NADPH oxidases increases proline accumulation during salt or mannitol stress in Arabidopsis thaliana. New Phytologist. 208(4), 1138, 2015.
13. GUPTA K., SENGUPTA A., CHAKRABORTY M., GUPTA B. Hydrogen peroxide and polyamines act as double edged swords in plant abiotic stress responses. Frontiers in plant science. 7, 1343, 2016.
14. MILLER G., SUZUKI N., CIFTCI-YILMAZ S., MITTLER R. Reactive oxygen species homeostasis and signalling during drought and salinity stresses. Plant, Cell Environ. 33(4), 453, 2010.
15. AHMAD P., NABI G., JELEEL C., UMAR S. Free radical production, oxidative damage and antioxidant defense mechanisms in plants under abiotic stress. Oxidative stress: role of antioxidants in plants. Studium Press, New Delhi. 19, 2011.
16. PAN J., ZHANG M., KONG X., et al. ZmMPK17, a novel maize group D MAP kinase gene, is involved in multiple stress responses. Planta. 235(4), 661, 2012.
17. MEJIA-TENIENTE L., DURÁN-FLORES F.D.D., CHAPA-OLIVER A., et al. Oxidative and molecular responses in Capsicum annuum L. after hydrogen peroxide, salicylic acid and chitosan foliar applications. Int. J. Mol. Sci. 14(5), 10178, 2013.
18. MEJIA-TENIENTE L., DURÁN-FLORES B. A., TORRES-PACHECO I., et al. Hydrogen peroxide protects pepper (Capsicum annuum L.) against pepper golden mosaic geminivirus (PeperGMV) infections. Physiological and Molecular Plant Pathology. 106, 23, 2019.
19. CLARK M. F., ADAMS A. Characteristics of the microplate method of enzyme-linked immunosorbent assay for the detection of plant viruses. J. Gen. Virol. 34(3), 475, 1977.
20. LI H., DING X., WANG C., et al. Control of tomato yellow leaf curl virus disease by Enterobacter asburiaeBQ9 as a result of priming plant resistance in tomatoes. Turkish Journal of Biology. 40(1), 150, 2016.
21. LICHTENTHALER H.K., BUSCHMANN C. Chlorophylls and carotenoids: Measurement and characterization by UV-VIS spectroscopy. Current protocols in food analytical chemistry. 2001.
22. ZHANG X., Research methodology of crop physiology. Agri. Press, Beijing, 208, 1992.
23. CHANDLLE J., SCANDALIOS J. Analysis of variants affecting the catalase developmental program in maize scutellum. Theoretical and Applied Genetics. 69(1), 71, 1984.
The Impact of Hydrogen Peroxide...

24. BEAUCHAMP C., FRIDOVIČ I. Superoxide dismutase: improved assays and an assay applicable to acrylamide gels. Anal. Biochem. 44 (1), 276, 1971.

25. ZHANG X. Research methodology of crop physiology. Agri. Press, Beijing. 208, 1992.

26. JANA S., CHOU DHURI M. A. Glycolate metabolism of three subserved aquatic angiosperms during ageing. Aquat. Bot. 12, 345, 1982.

27. SINGLETON V.L., ROSSI J.A. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. Am. J. Enology Viticult. 16 (3), 144, 1965.

28. SHIMADA K., FUJIKAWA K., YAHARA K., NAKAMURA T. Antioxidative properties of xanthan on the autoxidation of soybean oil in cyclodextrin emulsion. Journal of agricultural and food chemistry. 40 (6), 945, 1992.

29. LOWRY O.H., ROSEBROUGH N.J., FARR A.L., RANDALL R.J. Protein measurement with the Folin phenol reagent. J. Biol Chem. 193 (1), 265, 1951.

30. MOORE S., STEIN W.H. Photometric ninhydrin method for use in the chromatography of amino acids. Journal of biological chemistry. 176 (1), 367, 1948.

31. BATES L.S., WALDREN R.P., TEARE I. Rapid determination of free proline for water-stress studies. Plant Soil. 39 (1), 205, 1973.

32. GAO Q.-M., ZHU S., KACHROO P., KACHROO A. Signal regulators of systemic acquired resistance. Frontiers in plant science. 6, 228, 2015.

33. DUTTA H., KUMAR R.G., BORAHI M. Efficacy of Biotic and Chemical Inducers of SAR in Management of Plant Viruses. 2019.

34. CHOU DHURI F.K., RIVERO R.M., BLUMWALD E., MITTLER R. Reactive oxygen species, abiostress and stress combination. The Plant Journal. 90 (5), 556, 2017.

35. MEKONNEN A., DEGU Y., CARLSON R. Appraisal of solvent system effect on bioactivity profiling of Cordia africana stem bark extracts. Chemistry International. 6 (1), 1, 2020.

36. KHAN T.A., YUSUF M., FARIDUDDIN Q. Hydrogen peroxide in regulation of plant metabolism: Signalling and its effect under abiostress. Photosynthetica. 56 (4), 1237, 2018.

37. SMIRNOFF N., ARNAUD D. Hydrogen peroxide metabolism and functions in plants. New Phytol. 221 (3), 1197, 2019.

38. SAXENA I., SRIKANTH S., CHEN Z. Cross talk between H₂O₂ and interacting signal molecules under plant stress response. Frontiers in plant science. 7, 570, 2016.

39. ASHFAQE F., KHAN M.I.R., KHAN N.A. Exogenously applied H₂O₂ promotes proline accumulation, water relations, photosynthetic efficiency and growth of wheat (Triticum aestivum L.) under salt stress. Annual Research & Review in Biology. 105, 2014.

40. SHARMA I., AHMAD P. Catalase: a versatile antioxidant in plants. Oxidative Damage to Plants: Elsevierp. 131, 2014.

41. TERZI R., SARUHAN-GULER N., BISKIN N., KADIOGLU A. Exogenous Hydrogen Peroxide Alleviates Copper Toxicity by Stimulating Antioxidant System and Increases Photosynthesis Efficiency in Maize Seedlings. FEB-FRESENIUS ENVIRONMENTAL BULLETIN. 996, 2018.

42. SZÖLLÖSI R. Superoxide dismutase (SOD) and abiostress tolerance in plants: an overview. Oxidative Damage to Plants: Elsevierp. 89, 2014.

43. CLARKE S.F., GUY P.L., BURRITT D.J., JAMESON P.E. Changes in the activities of antioxidant enzymes in response to virus infection and hormone treatment. Physiol. Plant. 114 (2), 157, 2002.

44. RADM E.M., LUG, FAYEZ K.A., MAHMOUD S.Y. Protective action of salicylic acid against bean yellow mosaic virus infection in Vicia faba leaves. Journal of plant physiology. 165 (8), 845, 2008.

45. RADM E.M., FAYEZ K.A., MAHMOUD S.Y., HAMAD A., LU G. Salicylic acid alleviates growth inhibition and oxidative stress caused by zucchini yellow mosaic virus infection in Cucurbita pepo leaves. Physiological and Molecular Plant Pathology. 69 (4), 172, 2006.

46. RADM D., MOHAMED A., FAYEZ K., ABDELRAHMAN A. Oxidative stress caused by Basagran® herbicide is altered by salicylic acid treatments in peanut plants. Heliyon. 5 (5), e01791, 2019.

47. AZARABADI S., ABDOLLAHI H., TORABI M., SALEHI Z., NASIRI J. ROS generation, oxidative burst and dynamic expression profiles of ROS-scavenging enzymes of superoxide dismutase (SOD), catalase (CAT) and ascorbate peroxidase (APX) in response to Erwinia amylovora in pear (Prunus communis L). European Journal of Plant Pathology. 147 (2), 279, 2017.

48. SCHMID-SIEGERT E., STEPUHSENKO O., GLAUSER G., FARMER E.E. Membranes as structural antioxidants recycling of malondialdehyde to its source in oxidation-sensitive chloroplast fatty acids. Journal of Biological Chemistry. 291 (25), 13005, 2016.

49. RADM E.M., FAYEZ K.A., MAHMOUD S.Y., LU G. Modifications of antioxidant activity and protein composition of bean leaf due to Bean yellow mosaic virus infection and salicylic acid treatments. Acta Physiologica Plantarum. 32 (5), 891, 2010.

50. HUSEYNOVA L., MIRZAYEVA S., SULTANOVA N., ALIYEVA D., MUSTAFAYEV N.S., ALIYEV J. Virus-induced changes in photosynthetic parameters and peroxidase isozyme contents in tomato (Solanum lycopersicum L.) plants. Photosynthetica. 56 (3), 841, 2018.

51. ZHAO L., FENG C., WU K., et al. Advances and prospects in biogenic substances against plant virus: a review. Pestic. Biochem. Physiol. 135, 15, 2017.

52. EL HADRAMI A., ADAM L.R., EL HADRAMI L., DAAYF F. Chitosan in plant protection. Mar. Drugs. 8 (4), 968, 2010.

53. BEKTAS Y., EULGEM T. Synthetic plant defense elicitors. Frontiers in plant science. 5, 804, 2015.

54. LI X., AN M., XIA Z., BAI X., WU Y. Transcriptome analysis of watermelon (Citrullus lanatus) fruits in response to Cucumber green mottle mosaic virus (CGMMV) infection. Sci. Rep. 7 (1), 16747, 2017.

55. HILDEBRANDT T.M., NESI A.N., GRAU CO., BRAUN H.-P. Amino acid catabolism in plants. Molecular plant. 8 (11), 1563, 2015.

56. JOSHI R., RAMANARAO M.V., BAISAKH N. Arabidopsis plants constitutively overexpressing a myo-inositol 1-phosphate synthase gene (SaINO1) from the halophyte smooth cordgrass exhibits enhanced level of tolerance to salt stress. Plant Physiol. Biochem. 65, 61, 2013.

57. SORKHE K., SHIRAN B., KHODAMBASHI M., ROUHI V., MOSAHE M., SOFO A. Exogenous proline alleviates the effects of H₂O₂-induced oxidative stress in wild almond species. Russian journal of plant physiology. 59 (6), 788, 2012.
58. ZAYNAB M., FATIMA M., ABBAS S., et al. Role of secondary metabolites in plant defense against pathogens. Microb. Pathog., 2018.
59. BHATTACHARYA A., SOOD P., CITOVSKY V. The roles of plant phenolics in defence and communication during Agrobacterium and Rhizobium infection. Mol. Plant Pathol. 11 (5), 705, 2010.
60. SAED-MOUCHESHI A., PAKNIYAT H., PIRASTEH-ANOSHEH H., AZOOZ M. Role of ROS as signaling molecules in plants. Oxidative Damage to Plants: Elsevier pp. 585, 2014.
61. KARUPPANAPANDIAN T., MOON J.-C., KIM C., MANOHARAN K., KIM W. Reactive oxygen species in plants: their generation, signal transduction, and scavenging mechanisms. Australian Journal of Crop Science. 5 (6), 709, 2011.
62. GILL S.S., TUTEJA N. Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. Plant physiology and biochemistry. 48 (12), 909, 2010.
63. IMAHORI Y. Chapter 14 - Role of Ascorbate Peroxidase in Postharvest Treatments of Horticultural Crops. In: Ahmad P, ed. Oxidative Damage to Plants. San Diego: Academic Press pp. 425, 2014.