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Research article

Physiological and metabolic changes of *Cucurbita pepo* leaves in response to zucchini yellow mosaic virus (ZYMV) infection and salicylic acid treatments

Deya Eldeen Mohammed Radwan [a,b], Khalaf Ali Fayez [b], Sabry Younis Mahmoud [c], Ahmed Hamad [d], Guoquan Lu [a,*]

[a] *College of Agriculture & Biotechnology, Zhejiang University, 268 Kaixuan Road, Hangzhou 310029, Zhejiang, China*
[b] *Botany Department, Faculty of Science, Sohag University, Sohag 82524, Egypt*
[c] *Virology Department, Faculty of Agriculture, Sohag University, Sohag 82786, Egypt*
[d] *Botany Department, Faculty of Science, Assiut University, Assiut, Egypt*

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Abstract

The changes of some physiological and biochemical parameters in pumpkin (*Cucurbita pepo* cv Eskandarani) leaves associated with zucchini yellow mosaic virus (ZYMV) infection and the effect of exogenous application of salicylic acid (SA) were studied in this paper. In comparison to the untreated leaves, ZYMV infected leaves showed many symptoms, including severe mosaic, size reduction, stunting and deformation. Results from analysis of physiological parameters indicated that viral infection and SA treatments affected metabolism. Viral infection decreased pigment, protein and carbohydrate levels. But with all SA treatments, the protein and carbohydrate contents are noticeably increased. Moreover, the other biochemical parameters showed variable alterations. The peroxidase (POX, EC 1.11.1.7) activity and proline contents were induced by both viral infection and SA treatments. In addition, protein patterns represent some newly synthesized polypeptides which reflect formation of pathogenesis related proteins in all treatments. SA treatment increases the plant resistance against ZYMV. This can be noticed through reduction of percentage of the infected plants, decrease in disease severity and virus concentration of the plants treated with SA then inoculated with virus. All results show significant changes in metabolism affected by either viral infection or SA treatments and also indicate that exogenous SA plays an important role in induction of defense mechanism against ZYMV infection.

Keywords: Metabolism; Peroxidase; Pigments; Pumpkin; Salicylic acid; Systemic acquired resistance; Zucchini yellow mosaic virus

1. Introduction

Many types of environmental stresses both biotic and abiotic produce characteristic changes in physiology and metabolic processes of higher plants [26]. Among these stresses, attack by pathogens which cause many biochemical changes lead to harmful effects on plant health.

There are more than 20 viruses infecting cucurbit crops [8]. Zucchini yellow mosaic virus (ZYMV) is one of the most important viruses affect cucurbit production. It causes destructive diseases to a large variety of economically important cucurbit plants including zucchini squash (*Cucurbita pepo*) [44].

ZYMV was reported in many African countries including Algeria, Egypt, Madagascar, Mauritius, Morocco, Reunion, Swaziland and Tunisia [22]. Morphological symptoms

Abbreviations: Chl, chlorophyll; POX, peroxidase; SA, salicylic acid; SAR, systemic acquired resistance; SA + V, treated with salicylic acid then inoculated with virus; ZYMV, zucchini yellow mosaic virus.

* Corresponding author. Tel./fax: +86 571 8697 1117.
E-mail addresses: deya90@yahoo.com (D.E.M. Radwan), lugq10@zju.edu.cn (G. Lu).

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observed on leaves are vein clearing, yellowing, mosaic, and deformations of leaves [8]. The disease can completely destroy the crop under favorable conditions leading to yield loss.

It was reported that plant viruses affect physiological processes such as photosynthesis [26] by decreasing the photosynthesis rate (depending on the infection stage), decreasing pigment contents [28], soluble sugar contents, reducing starch accumulation [35], and increasing the respiration rates [33]. Técsi et al. [35] reported changes in host metabolism in relation to virus replication in the infected marrow cotyledons. Some enzymes are widely influenced in response to pathogen attack [42]. Among these enzymes, Peroxidase is the first to show changes in its activity during viral infection stages. For instance, POX shows increase in its activity in *Cucurbita pepo* plants infected with viruses such as CMV [35].

SAR is considered a form of resistance induced in plants against subsequent infection and attack by a broad spectrum of organisms [1,11]. Several chemicals have been reported as SAR inducers in plants [36]. SA is considered one of the key components of defense signal transduction which induces the full set of SAR genes [23]. Also, SA is known as a regulator for physiological processes such as plant defence mechanisms against harmful microorganisms.

Application of SA led to H_2O_2 accumulation and may include the induction of at least one of cellular protective mechanisms that are concomitant with the accumulation of active oxygen species suggesting that SA application results in a state of oxidative stress [27]. Therefore, it can be predicted that SA may affect numerous metabolic processes in plants [25] and through the potentiation of oxidative burst; SA can control both biotic and abiotic defense programs [3]. For this, we found that it is important to study the effects of SA as well as SA followed by virus inoculation.

The term systemic acquired resistance refers to a change in the physiology of the plant [31]. There is a number of biochemical and physiological changes that have been established to be associated with SAR, which include cell death and oxidative burst [20], deposition of lignin [37], accumulation of proline [13] the synthesis of phytoalexins [4,29] accumulation of pathogenesis-related proteins [16] changes in pigments content, chlorophyll synthesis and POX activity [28].

In this paper, we investigate effects of ZYMV infection, SA and (SA + V) treatments on some physiological and biochemical parameters in pumpkin (*Cucurbita pepo*) leaves. Specifically, the analyses are of proteins, pigments and carbohydrate contents, POX activity and the protein profile by SDS-PAGE also indicate the induction of resistance by SA treatments against ZYMV infection.

2. Materials and methods

2.1. Plant materials

Seeds of pumpkin (*C. pepo* cv Eskandarani) were sown in a mixture of sand and clay (1:2 v/v) in plastic pots (10 cm in diameter) in separated growth chambers with a photoperiod of 12 h. Day and night temperatures were 24 °C and 18 °C, respectively; the relative humidity was about 70%. The plants were kept at 100% relative water content.

2.2. SA treatments and virus inoculation

After 21 days of growth, plants were divided into eight groups. Each group consists of three replications. One group was left as a control without any treatment. The other groups were treated with 10, 50 and 100 μM of SA by spraying the leaves until run-off. The control was sprayed by water. To improve spread, two drops of Tween 80 were added. Inoculation of virus was performed 3 days after spraying. All leaves were mechanically inoculated. The inoculum was prepared from infected top leaves, ground in a mortar containing 0.1 M phosphate buffer pH 7.0 (1:2 w/v); the homogenate was filtrated through two layers of muslin, and the leaves of healthy plants were dusted with carborundum, and rubbed gently with cotton swab previously dipped into the suspension of virus inoculum. 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 formula according to Yang et al. [43]:

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

Three weeks after inoculation the youngest fully developed leaves from both control and treated plants were collected for analysis of biochemical changes.

2.3. ZYMV detection

2.3.1. DAS-ELISA

DAS-ELISA Technique was applied as described by Clarke and Adams [6] for ZYMV concentrations in the infected and (SA + V) treated leaves using an ELISA Kit (completely ready for use). Kits were supplied by SANOFI, Sante Animale, Paris, France.

2.3.2. Electron microscopy

Leaf pieces developing severe symptoms were ground in a drop of PBS pH 7 + 0.01% (w/v) sodium sulphite (Na_2SO_3). The leaf extracts were then transferred to carbon-coated Formvar grids for seven minutes. After washing with distilled water, the grids were negatively stained with 2% uranyl acetate and examined with a JEOL 1220 transmission electron microscope.

2.4. Determination of photosynthetic pigments

Contents of Chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*) and total carotenoids were spectrophotometrically determined according to Metzner et al. [24]. The pigment contents of those infected, treated with SA and inoculated after treatment