Physiological and Molecular Plant Pathology 96 (2016) 47–59

Contents lists available at ScienceDirect

Physiological and Molecular Plant Pathology

journal homepage: www.elsevier.com/locate/pmpp

Exogenous salicylic acid treatment delays initial infection and counteracts alterations induced by *Maize dwarf mosaic virus* in the maize proteome

I.A. Cueto-Ginzo [a, c], L. Serrano [b], E. Sin [b], R. Rodríguez [a], J.G. Morales [d], S.B. Lade [c], V. Medina [a, b, *], M.A. Achon [a, b]

[a] Grupo INPLAMICVEC, Departament de Producció Vegetal i Ciència Forestal, Universitat de Lleida (UdL), Avda. Rovira Roure 191, 25198, Lleida, Spain
[b] AGROTECNIO Center, Universitat de Lleida, Avda. Rovira Roure 191, 25198, Lleida, Spain
[c] UdL-IMPULS Programme, Universitat de Lleida, Avda. Rovira Roure 191, 25198, Lleida, Spain
[d] Facultad de Ciencias Agrarias, Departamento de Ciencias Agronómicas, Universidad Nacional de Colombia, sede Medellín (UNALMED), calle 59 A N 63-20, Bloque 11, Núcleo El Volador, Medellín, Colombia

ARTICLE INFO

Article history:
Received 22 February 2016
Received in revised form
16 June 2016
Accepted 25 July 2016
Available online 27 July 2016

Keywords:
Salicylic acid
Zea mays
Maize dwarf mosaic virus
Proteomics

ABSTRACT

The phytohormone salicylic acid (SA) was applied to healthy and *Maize dwarf mosaic virus* (MDMV)-infected maize plants, and differential proteomic analysis was simultaneously performed to evaluate the physiological effects and the SA-maize-virus interaction in the early pathogenesis cycle. MDMV infection reduced photosynthetic rates, while SA increased them, together with plant height and roots in healthy plants. In infected plants, SA increased RuBisCo and stabilized chlorophyll a-b binding protein expression, increased plant height and root volume, delayed symptom expression, and reduced infection rate. Results highlight biochemical pathways involved in the maize-MDMV pathosystem and signal SA as key to maize disease management programs.

1. Introduction

The exogenous application of salicylic acid (SA) has variable and positive effects on a wide range of physiological processes in different crops. In general, effects induced by SA are related to diminished disease development or boosted plant immune responses under biotic and abiotic stress. Particularly in maize plants, SA reduces chilling injury [26], mitigates the adverse effects of drought and salinity [17,45], increases polyamine content [38], alleviates oxidative stress [43], and decreases the toxic effect of cadmium on photosynthesis [3]. On the cellular level, SA is known to be an important regulator of photosynthesis because it affects leaf and chloroplast structure [55], the activity of enzymes such as ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO) [51], chlorophyll and carotenoid contents [18], and stomatal closure [34]. In turn, the endogenous synthesis of SA may be induced in response to environmental stresses, which trigger Systemic Acquired Resistance (SAR) [57].

During recent years, available information regarding SA and plant virus control has become more prolific [5]. The importance of SA arises from its role in the mediation of (R)-gene resistance and basal immune responses; and the positive link between SA-mediated defense and the small interfering RNA (siRNA) antiviral machinery [2]. It has been well established that SA can interfere in three main stages of the virus pathogenicity cycle including replication, cell to cell movement and long distance movement [52].

Maize dwarf mosaic virus (MDMV) is a potyvirus with worldwide distribution most common to tropical regions, causing severe losses in maize and sorghum crops [50]. It is transmitted predominantly by several genera of aphids (both adults and nymphs) in a non-persistent manner, but also mechanically and via seeds in a very low proportion [20]. According to CIMMYT [7], MDMV-infected plants develop distinct chlorotic mosaics, mottles or streaks on green tissues (typically observed in young leaves) and show stunting and shortening of upper internodes, increased tillering and a poor seed set. These symptoms vary widely depending on

* Corresponding author. Grupo INPLAMICVEC, Departament de Producció Vegetal i Ciència Forestal, Universitat de Lleida (UdL), Avda. Rovira Roure 191, 25198, Lleida, Spain.
E-mail address: medinap@pvcf.udl.cat (V. Medina).

http://dx.doi.org/10.1016/j.pmpp.2016.07.001
0885-5765/

host genotype, time of infection, and viral strain.

For the efficient control of MDMV (Sp strain) using SA-dependent methods, a more complete understanding of its interactions with the different hosts and plant-associated resistance mechanisms at the molecular level is necessary, especially since the effects of the continuous application of various SA doses on seedlings and plants is poorly understood. In this context, we have tested the exogenous treatment of MDMV-infected plants with SA, basing our study on previously reports citing success with symptom control of other viruses in maize like *Maize rough dwarf virus* (MRDV) [56], and *Potato virus X* (PVX) in tomato [36].

The efficiency of exogenous SA application depends on multiple factors such as the plant species, developmental stage of the plant, application methods, and SA concentration [6]; hence the importance of accurately testing the effects of SA on each pathosystem. In the present study, we examine the biological relevance of exogenous salicylic acid (SA) treatment in the MDMV-Sp/maize pathosystem, exploring the possibility for its employment in MDMV control. In order to better comprehend the effect of SA on maize physiology, we investigated the variation of net photosynthetic rate, gas exchange properties and growth parameters in maize plants infected, or not, by MDMV-Sp and treated with different doses of SA at regular intervals. In addition, a differential proteomic analysis was performed during the initial phase of viral infection to gain a deeper understanding of the plant-virus interaction.

2. Material and methods

2.1. Plant material, MDMV strain and growth conditions

Maize cv. B73 seeds were sown in pots containing autoclaved commercial substrate (Traysubstrat®, Klasmann-Deilmann, Gmbh, Geeste, Germany). Plants were then grown and maintained under greenhouse conditions, at 18–25% humidity, 25–30 °C, with supplementary lighting (18 h photoperiod), automated irrigation and protected from insect attack by anti-aphid mesh. The virus strain MDMV-Sp (accession number, AM110558), endemic to Spain, was used. This strain was maintained in maize plants (cv. B73) in the greenhouse by continuous mechanical inoculation.

2.2. Exogenous SA application and MDMV inoculation

Sampling times and timelines for SA treatments, virus-inoculation and each analysis are detailed in Fig. 1.

Two different dilutions in sterile distilled water of commercial SA (Rhodia, France) were used: 0.5 mM and 5 mM (SA1 & SA2). These doses were selected based on results of several previous studies (see revision by Manzoor et al.) [33]. The first SA application was administered two days before MDMV-Sp-inoculation by spraying all leaves until run-off was observed. Mechanical inoculation of the virus strain using carborundum (600-mesh) was carried out at the phenological state of three fully expanded leaves, two days after the first SA treatment. The inoculum was prepared by triturating young MDMV-Sp-infected leaves (from plants inoculated 12–30 days earlier) with a mortar and pestle in 2 vol of 0.05 M Na-K phosphate buffer pH 7, and applied by gentle rubbing with a finger. SA dilutions were sprayed three more times (at 7 day intervals), for a total of four applications.

A total of 20 plants were used per treatment. This number was selected based on a previous study citing it as sufficient for obtaining the necessary quantity of leaf material needed for the ELISA tests and proteomic analysis. At 21 days, plants of similar size were selected and divided into different groups consisting of four replications (each replication was one pot containing five healthy plants). Groups were treated as follows:

Group 1: healthy control, plants sprayed with water.
Group 2: infected control, plants inoculated with MDMV-Sp at the same time as other test groups.
Groups 3 and 4: SA1 and SA2 plants treated with 0.5 and 5 mM SA, respectively, by spraying all leaves until run-off was observed.
Groups 5 and 6: SA1+V and SA2+V, plants treated with 0.5 and 5 mM SA, respectively, and MDMV-Sp inoculated, as described above.

2.3. Symptom expression and physiological parameter analysis

Post inoculation, plants were periodically tested for the presence of mosaic and chlorotic streak symptoms (see sampling calendar, Fig. 1). Measurements of gas exchange parameters were obtained with an ADC LCA4 infrared gas analyzer (ADC Bioscientific, England) under ambient light conditions. The active photosynthetic radiation measured was 150–300 mmol m^{-2} s^{-1} in the greenhouse and an average of three measurements were taken per leaf (per plant). To indicate leaf chlorophyll content, a SPAD meter was used (Minolta SPAD-502, Konica Minolta Ltd), to record 3 measurements per leaf, at 3 leaves per plant. The measurement timeline was performed according to Fig. 1.

2.4. Root analysis

The roots of 3 plants per treatment from each sampling date were examined (Fig. 1). Roots were carefully washed with tap water to avoid tissue damage, and preserved in 50% ethanol (w/w) at 4 °C until further analysis. Root images were obtained with an Epson Perfection V700 modified flatbed scanner. Roots were thoroughly analyzed, accounting for length, volume, surface area, number of tips, forks and average diameter using WinRHIZO software (version 2009; Regent Instruments Inc., Quebec, ON, Canada). The parameters proving to be most representative of growth fluctuations would be used for the results and comparative analysis.

2.5. ELISA test

To confirm and estimate the infection, a standard indirect ELISA following the standard Clark & Bar-Joseph method [8] was performed every week after the mechanical inoculation of the virus strain in all samples (leaf tissue) of groups 5 and 6, following the sampling calendar (Fig. 1). The antiserum used for diagnosis was of commercial origin and specific to the MDMV-Sp strain that was tested in the laboratory with satisfactory results (MDMV-Sp, 07059 Loewe Biochemica GmbH, polyclonal). Plants were considered infected when the absorbance for positive samples was at least two times the negative control value (non-inoculated plant). Absorbance values were measured at 405 nm using a Microplate Spectrophotometer (Biorad, model 680).

2.6. Protein extraction

Protein analysis was only conducted for plants treated with the higher, 5 mM dose of salicylic acid (SA2), as this dose showed pronounced effects on viral infection according to ELISA results. Thus, samples referred to in this section are Control (C) plants, plants treated with 5 mM of salicylic acid (SA2), plants infected with MDMV-Sp (V), and plants treated with both 5 mM salicylic acid and infected with MDMV-Sp (SA2+V). Samples were taken periodically after repeated SA treatment. T1, T2, T6 and T8 indicate sampling times as follows: T1 (0 days, directly after the 1st treatment), T2 (2 days post 1st treatment), T6 (2 days post 3rd