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ARTICLE

The beneficial effects of S-methyl-methionine in maize in the case of *Maize dwarf mosaic virus* infection

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ABSTRACT *Maize dwarf mosaic virus* (MDMV) is one of the most common pathogens infecting maize plants. Its infection causes decreased plant growth and chlorotic bands on the leaves giving a mosaic pattern. Our efforts aimed to increase the plants' inherent resistance with exogenic addition of the natural compound S-methyl-methionine (SMM) that plays a central role in the plant sulphur metabolism. SMM is a key compound in various metabolic pathways connected with resistance mechanisms of several stresses. In the present study Jubilee sweet corns were used, on which the harmful effects of MDMV and the advantageous actions of SMM were recorded applying non-invasive fluorescence imaging and induction methods and DAS-ELISA test. According to the results, the SMM treatment improved the physiological parameters of the maize plants (including photosynthetic rate and the amount of chlorophyll pigments), and nevertheless it significantly improved the plants' defence response to viral infection.
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KEY WORDS

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Maize (*Zea mays* L.) is one of the most widely grown crops worldwide. Due to its importance as a food and feed plant, investigations on tolerance to pathogens and attempts to find alternative methods to increase its defence potential are of great importance.

The common maize pathogen *Maize dwarf mosaic virus* (MDMV) spreads in natural or in agro-environment via aphids, or by seed transmission. In the development of epidemics of the MDMV disease the susceptible reservoir weed plant *Sorghum halepense* plays a significant role (Tóbiás et al. 2008). The MDMV is a member of the *Potyvirus* genus in the *Potyviridae* family. The virus has a single-stranded positive-sense RNA genome. The symptoms of the infection are reduced growth (root-shoot ratio), and "chlorotic" bands on the leaves, which are thought to be the result of RNA induced silencing mechanism (Wadsworth and Dunoyer 2009). Along the pale green-yellow streaks on the leaves, the virus is present, while in the darker green parts, due to the local silencing, the virus is absent. This duality results in a mosaic pattern (Astier et al. 2007).

For the improvement of tolerance to biotic and abiotic stressors, the application of biologically active compounds - with a favourable effect on physiological processes resulting in an increase in defence potential - seems to be an alternative possibility. S-methyl-methionine (SMM), a non-proteinogenic sulphur containing amino acid, is one such naturally occurring biologically active compound (Rácz et al. 2008). SMM is synthesised from methionine, and can be converted back to methionine, resulting in a circular pathway, known as the SMM-cycle. SMM plays a role in the regulation of methionine and S-adenosyl-methionine levels, is involved in the methylation processes taking place in cells and is an important compound for the transport and storage of sulphur (Bourgis et al. 1999). The complexity of its functions indicates that SMM plays a wide-ranging regulatory role in plant physiological processes. The SMM contributes to the increase of resistance, as it is a direct precursor of the osmoprotectant sulphopropionates and other S-containing compounds involved in defence mechanisms, while also influencing the biosynthesis of plant hormones such as polyamines and ethylene (Rácz et al. 2008). When stress factors are present, the up-regulation of the phenylpropanoid biosynthetic pathway is detected, that contributes to the production of certain fenoloids, flavonoids and anthocyanins, which compounds are characterised by antioxidant quality and show higher absorbance in the UV spectrum. Exogenous SMM also increases the membrane stability and the activity of enzymes that eliminate reactive oxygen species (Kósa et al. 2011).

In the present study the effects of SMM on maize MDMV infection process and response reactions were studied using non invasive fluorescence imaging and chlorophyll-a fluorescence induction methods and DAS-ELISA test. The main aim of this work was to give new data and contribute to our knowledge and better understanding on the beneficial effects of SMM in the course of MDMV infection.

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Materials and methods

Plant growth

Zea mays 'Jubilee' plants were grown on ¼ strength Hoagland solution in growing chamber PGR-15 (Conviron, Canada) with a 14/10 h light/dark period and a light intensity of 300 $\mu Em^{-2}\ s^{-1}$, a day/night temperature of 25/22°C and 70% relative humidity. To study the effect of SMM 9-day-old plants were placed in ¼ strength Hoagland solution containing 0.01g/L SMM for 24 h. The MDMV infection was carried out at the tenth day. The first leaves of the plants were inoculated mechanically with MDMV-Dallas strain. Leaves from infected plants showing macroscopic symptoms were homogenised in Sörensen phosphate buffer (1g/5ml w/v) and used for inoculation. Celit powder was added as abrasive.

Multispectral fluorescence imaging

The measurements were carried out using FL-FIS (compact flash-lamp fluorescence imaging system) apparatus, which contained a pulsed xenon lamp, that flashed with 16,7 Hz and had a UV transmission filter with a maximum at 355 nm. The multispectral fluorescence images for the blue (F440), green (F520), red (F690) and far-red (F740) fluorescence were acquired with a CCD camera synchronously gated with the flash lamp (Buschmann et al. 2000). The apparatus' precise work can be read in Szigeti's (2008) article. Images were processed and F440/F690 and F690/F740 ratios were produced by Camille 1.05 software (Photonetics, Kehl, Germany). The raw image was corrected for nonuniform excitation with the pixels of a blue fluorescence image of a white paper showing uniform fluorescence, and for filter sensitivity. False-colouring was carried out with the ImageJ software package (http://rsb.info.nih.gov/ij).

Chlorophyll-a fluorescence induction measurements

Determination of variable fluorescence (Fv/Fm) values: Functional activity was determined in terms of variable fluorescence using a PAM fluorometer (Walz, Effeltrich, Germany). Samples consisted of 12 leaf discs from three plants, and measurements were made after 20 min dark adaptation.

DAS-ELISA test

DAS-ELISA (double antibody sandwich- enzyme linked immunosorbent assay) test was applied for determining the concentration of MDMV present in the leaves. Bioreba MDMV antiserum kit was used for the detection of the virus. The intensity of the colour was measured at 405 nm with Labsystem Multiscan MS spectrophotometer (ELISA reader).

Results and discussion

Based on previous results remarkable information has already been available on the favourable effect of exogenous SMM, which is manifested in the increased stress tolerance of plants exposed to various stressors (cold, drought, biotic stress factors) (Kósa et al. 2011). These effects can probably be explained partly by the direct role of SMM in the S metabolism of plants and partly by indirect effects, such as its influence on the metabolism of plant hormones which play a complex regulatory role (Rácz et al. 2008).

In the present work we investigated the stress response reactions of maize plants infected with MDMV-Dallas virus at the age of 10 days, as compared to the reactions of infected plants that had SMM treatment prior to infection, monitoring the spreading of MDMV during a three-week period after virus inoculation.

Non-invasive methods make it possible to detect fluorescence from the whole surface of the leaf, and analyse even single leaf areas specifically. From the most frequently analysed fluorescence regions the blue (F440), green (F520) fluorescence is mainly due to derivatives of ferulic acid, localised in the cell wall, and to certain cinnamoids and flavonoids that are stored in cell vacuoles (Buschmann 1998). Red (F690) and far red (F740) emissions are derived from the PS II, more specifically from the chlorophyll-a pigments localised in the antenna region, however in the case of far red, the chlorophyll-a pigments from PS I further contribute to its value. The chlorophyll pigments in vivo show maximum absorption at 680 nm, which region greatly overlap with the emission spectrum of the red region (690 nm). As a consequence, as the amount of the chlorophyll pigments increase, the intensity level of the emission in the red region decreases. Therefore the ratio of F690/F740 is inversely proportional with the amount of chlorophyll localised in the leaves. The blue fluorescence is also reabsorbed to some extent in the actively photosynthesising tissues.

Symptoms of infection and differences between treatments can clearly be demonstrated after 10 days of inoculation at the 4th leaves of plants, these results are illustrated in Figure 1 and Table 1. According to the results, the intensity of the emitted fluorescence at 440 and 520 nm increased when plants were treated by SMM, which indicates that the amount of phenoloids slightly increased in these plants. Furthermore, the value of the F690/F740 ratio decreased, indicating the increase in chlorophyll content in the leaves. MDMV-infected plants also had increased amount of phenoloids (high intensity at 440 and 520 nm), however, the amount of chlorophyll pigments present in the leaves significantly decreased (as the increased F690/F740 ratio shows). When the infected plants had SMM treatment prior to the infection, the amount of the chlorophyll pigments did not change significantly.

Similar results were achieved with chlorophyll-a fluorescence induction measurements. The Fv/Fm value, that indicates the physiological status of the plant (more specifically the maximal quantum efficiency of PS II), decreased in the

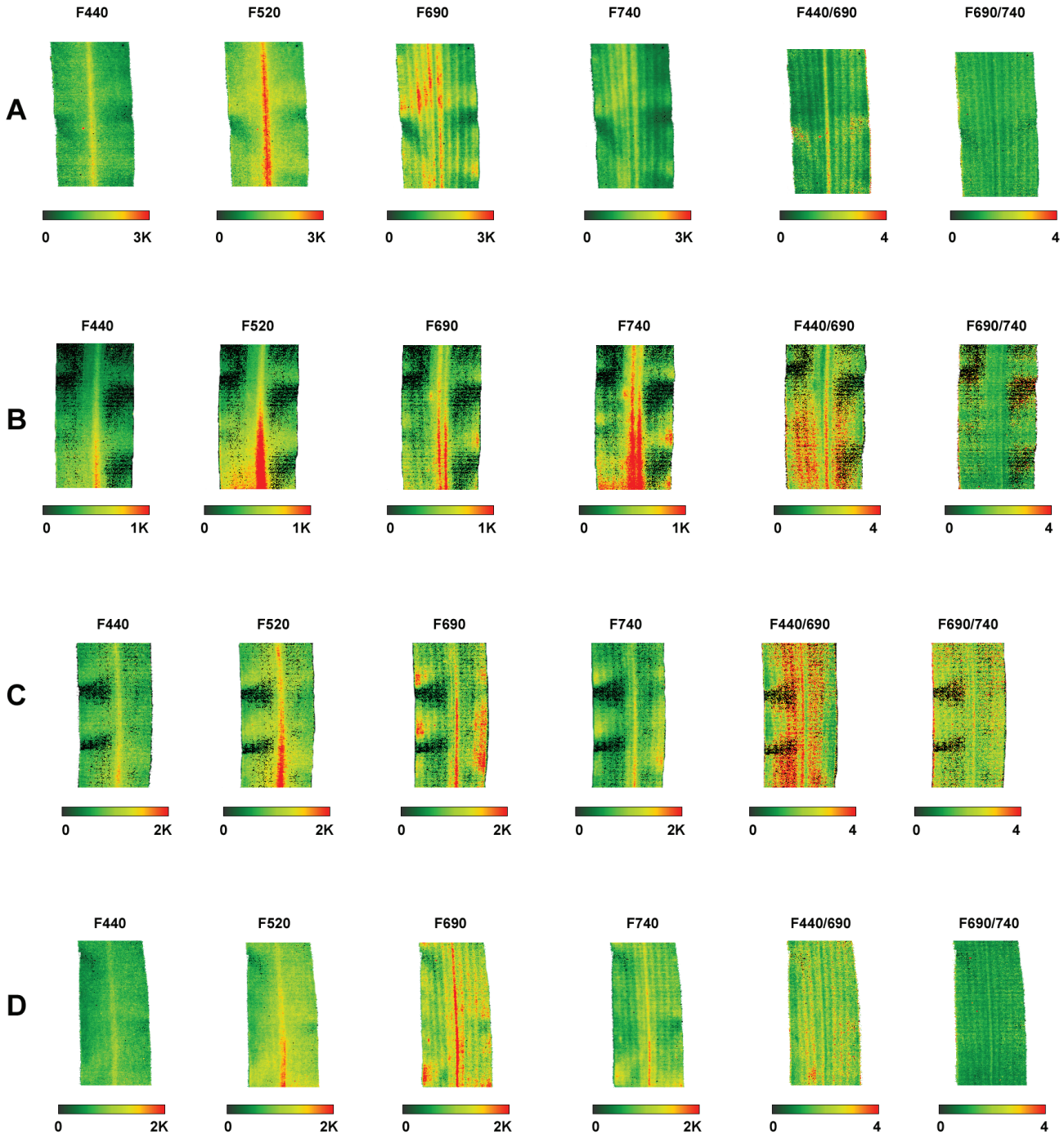

Figure 1. Fluorescence images of 4th leaves of maize plants after 10 days of inoculation taken in the blue (F440), green (F520), red (F690), far-red (F740) spectral regions and blue/red (F440/F690) and red/far-red (F690/F740) ratios. The colours are false colours indicating the intensity of the emitted fluorescence: green < yellow < orange < red. A: control; B: control and SMM-treated; C: infected; D: infected and SMM-treated.

case of infected plants. However, when the infected plants also got SMM treatment prior to the infection, there was no significant change detected, as compared to control plants, indicating a protecting effect of SMM treatment for the plants prior to the stress of MDMV infection.

Infected plants, previously treated with SMM contained virus particles in significantly smaller concentration, as compared to the non treated plants, according to the ELISA results. These differences were even more pronounced after a longer period following to treatment as can be seen in Fig-

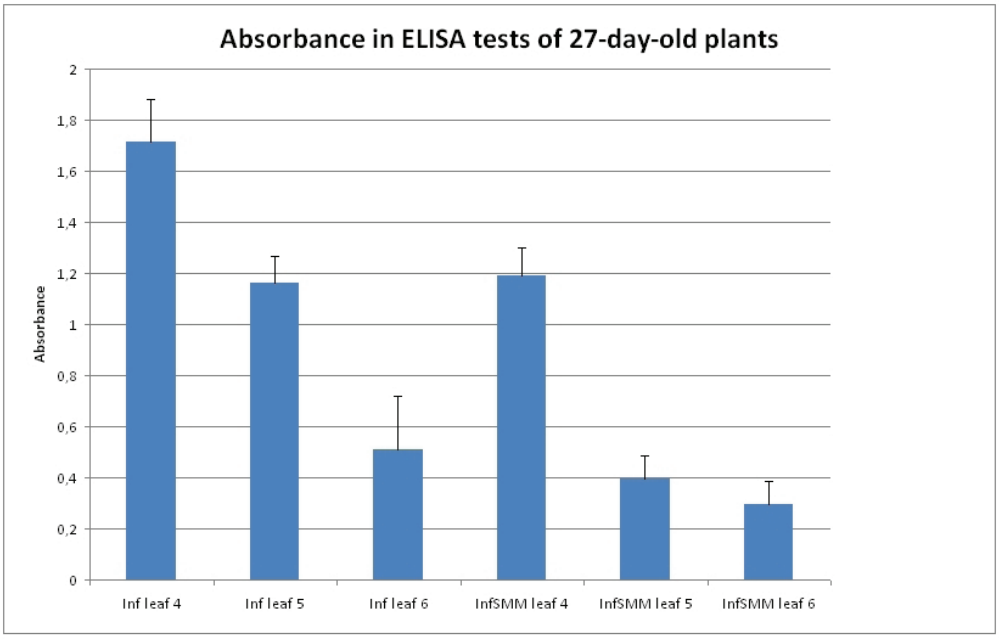

Figure 2. Absorbance units measured in ELISA tests of 27-day-old infected maize plants with or without SMM-treatment. 'Inf': infected; 'Inf-SMM': infected and SMM-treated.

Table 1. Average fluorescence intensity values on the measured wavelengths and ratios in the control and infected plants, with or without SMM treatment.

Wavelength (nm)	Control	SMM	Infected	Infected + SMM
440	417	507	503	402
520	654	795	694	557
690	455	430	899	1060
740	578	567	1072	1441
F440/F690	0,962	1,148	0,539	0,716
F690/F740	0,794	0,769	0,880	0,730

ure 2. Results of our experiments on the effect of exogenous SMM on fluorescence parameters indicating the amount of stress-protecting compounds, chlorophyll content and function of photosynthetic apparatus clearly demonstrates, that the natural compound SMM have beneficial effect on stress response reactions resulting in increase in defence potential of maize plants during MDMV infection.

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