**The protective role of SMM against Dwarf Mosaic Virus
infection in maize**

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**Abstract**

In this study we examined the effects of *S-*methylmethionine (SMM) on *Maize dwarf mosaic virus* (MDMV) infection in maize. We monitored changes in chlorophyll content, in the amount of viral coat protein and in the expression patterns of stress-related genes, such as *S-adenosylmethionine synthase* (*SAMS*) and a 14-3-3-like protein gene (*GF14-6*). A considerable decrease in chlorophyll content was observed in infected plants, but this was tempered by SMM pretreatment. The results of ELISA test showed that the amount of viral coat protein remained significantly lower in the leaves of infected plants pretreated with SMM. SMM was found to cause a considerable change in the expression patterns of the investigated genes previously proved to relate to MDMV infection.

Keywords: GF14-6, MDMV, SAMS, SMM, sweet corn

**Összefoglalás**

Kutatásunk során arra kerestük a választ, hogy milyen módon lehetne javítani a növény védekezőképességét és ezáltal visszaszorítani a vírus terjedését. Ennek vizsgálatához egy természetes biogén vegyülettel, az S-metilmetioninnal kezeltük a növényeket, majd ezt követően kukorica csíkos mozaik vírussal (MDMV) fertőztük a kukoricákat. Vizsgáltuk a klorofilltartalom változásait és két, a stresszválaszban szerepet játszó gén, a *GF14-6* (G-boksz faktor 14-3-3 homológ), és a *SAMS* (S-adenozilmetionin-szintáz) expressziójának változásait. Nyomon követtük továbbá a vírus mennyiségének a változását növényekben a kezelések hatására DAS-ELISA technikával. Megállapítottuk, hogy fertőzött növényekben csökken a klorofilltartalom, mely csökkenés SMM-előkezeléssel kivédhető. A vírusfertőzés és SMM-kezelés egyaránt befolyásolta a vizsgált gének expressziós változásait, mellyel összefüggésben a kombinált kezelés hatására csökkent a víruspartikulumok mennyisége a növényekben. Összességében elmondható, hogy kísérleteink során sikerült igazolnunk az S-metilmetionin védő hatását.

Kulcsszavak: csemegekukorica, GF14-6, MDMV, SAMS, SMM

**Introduction**

Maize (*Zea mays* L.) is one of the most widely cultivated crops worldwide. The need to improve its stress and disease tolerance is an urgent problem in agriculture. *Maize dwarf mosaic virus* (MDMV), a member of the *Potyvirus* genus, is one of the most important biotic stressors of sweet corn cultivars. The infection usually causes crop losses of 10-45%, but the damage may be as high as 100%. The virus preferentially colonizes members of the Poaceae family and spreads in natural or agro-environments via aphid, pollen and seed transmission (Tóbiás et al., 2008).

The use of a biologically active compound could be a feasible way to improve tolerance to certain abiotic and biotic stress factors. *S-*methylmethionine (SMM; (CH3)2-S-(CH2)2-CH(NH2)-COOH) occurs naturally in the plant kingdom as a non-proteinogenic, sulphur-containing amino acid. In plants, SMM is synthesised from methionine and can also be reconverted, resulting in a circular pathway, known as the SMM-cycle. SMM contributes to an increase in resistance, as it is a direct precursor of sulphur-containing compounds involved in defence mechanisms, while also influencing the biosynthesis of certain plant hormones (Rácz et al., 2008).

The present study investigated the effects of SMM on MDMV infection and on the stress response of maize. Chlorophyll content and the relative quantity of viral particles were assayed in infected leaves along with the expression patterns of genes involved in plant defence pathways.

**Materials and methods**

*Plant growth conditions:* Maize (*Zea mays* cv. *saccharata* Koern., sweet corn) plants were grown on ¼ strength Hoagland solution in SANYO MLR-350 HT growth chambers, with a 14/10 h light/dark period and a light intensity of 300 µmol/m2s, a day/night temperature of 25/22 °C and 70% relative humidity.

*Treatments:* To study the effects of SMM, 11-day-old plants were placed in ¼ strength Hoagland solution containing 2 mg/l SMM for 24 h. MDMV infection was carried out on the 12th and 14th days. The first and second leaves of the plants were inoculated mechanically with Dallas-A strain of MDMV.

*Measurement of chlorophyll content:* Chlorophyll extraction was carried out using 80% acetone, with subsequent photometric assays and calculations as described by Porra et al. (1989).

*cDNA synthesis and quantitative real-time PCR:* A ZR Plant RNA MiniPrep TM 2024 kit (Zymo Research, Irvine, CA, USA) was used for RNA extraction, and a First Strand cDNA Synthesis Kit (Thermo Scientific, Rockford, IL, USA) for cDNA synthesis, based on the manufacturers’ instructions. qRT-PCR measurements were carried out using a Power SYBR® Green PCR Master Mix (Life Technologies, Foster, CA, USA). The experiments were run on an ABI Prism® 7000 Sequence Detection System (Applied Biosystems, Foster, CA, USA). A maize *actin* gene and the membrane protein gene *PB1A10.07c* (*MEP*) were included in the assays as internal controls, and a 14-3-3-like protein GF14-6 and S-adenosylmethionine synthase (SAMS) were the genes of interest. The relative changes in gene expression were quantified according to the modified ΔΔCt method of Pfaffl (2001), where E levels are taken into account.

*DAS-ELISA test:* DAS-ELISA (double antibody sandwich - enzyme linked immunosorbent assay) was applied to detect MDMV coat protein in maize leaves, using an MDMV antiserum kit (Bioreba A.G., Reinach, Switzerland) following the manufacturer’s instructions. Absorbance (colour intensity) was measured at 405 nm with a Labsystem Multiskan MS spectrophotometer (Thermo Scientific, Rockford, IL, USA).

*Statistical data analysis:* The results were evaluated using analysis of variance (ANOVA), while Student’s *t* test was performed on the relevant data using IBM® SPSS® statistical software (version 20).

**Results**

The amount of chlorophyll molecules increased in SMM-treated (*S)* plants. By contrast, the chlorophyll content decreased in infected (*inf)* plants. The differences were most pronounced three weeks after the treatments. SMM-pretreated and infected (*Sinf)* plants and *S* plants contained 35.9 ± 2.5 and 35.4 ± 2.5 µg/cm2 chlorophyll, respectively, while control and *inf* plants contained 33.7 ± 2.4 and 24.3 ± 1.7 µg/cm2 respectively.

The gene expression levels of *GF14-6* increased in *S* and *inf* plants in the 1st and 3rd week after treatment. However, in the 2nd week all the treatments resulted in significant decreases. By contrast, in *Sinf* plants gene expression did not change in the 1st week as compared to the control, but dropped in the 3rd week. One week after treatment *inf* plants showed a 391% increase in the gene expression of *SAMS* compared to the control, but in the following weeks, the rate of gene expression declined. In *Sinf* plants a similar expression pattern was observed, though a week after treatment the gene expression level was still only 156% of the control in these plants. Interestingly, in the second and third weeks a pronounced increase in *SAMS* expression could be observed (187 and 219% of the control, respectively), indicating the possible conditioning effect of SMM in infected plants. In *S* plants no such pattern was observed.

The results of ELISA showed that *Sinf* plants contained significantly smaller amounts of MDMV coat protein than *inf* plants, and these differences increased with time after infection. Younger leaves had a lower concentration of MDMV particles than older leaves.

**Discussion**

A decrease in chlorophyll content was recorded in *inf* plants, however, SMM pretreatment increased the amount of chlorophyll molecules, and alleviated the decrease exerted by the infection. Our results demonstrated that SMM triggers chlorophyll biosynthesis indirectly, and based on previous results also protects biological membranes, resulting in a higher level of chlorophyll in the leaves.

In the present work MDMV infection differentially triggered the expression of *GF14-6* and *SAMS* genes. In *inf* plants, increases of 134% and 391% were detected for *GF14-6* and *SAMS,* respectively, in the first week, compared to the control plants. In the second week, the expression rates of both genes dropped, especially that of *GF14-6*. In the case of *SAMS*, gene expression further decreased in the third week. In *Sinf* plants low levels of *GF14-6* gene activity were measured. However, in *S* plants, high levels of gene expression were recorded in the first and third weeks. These data indicate that SMM treatment and MDMV infection trigger pathways that are related to high levels of *GF14-6* expression, but when the treatments are applied at the same time, the inhibition of *GF14-6* expression can be observed. In the case of *SAMS*, the expression level of *Sinf* plants was 156% in the first week compared to the control plants, which slightly increased in the second and third weeks. SMM pretreatment delayed the increase in *SAMS* expression in infected plants, but also prolonged it, providing a slow, but constant rise. This phenomenon could be related to improved virus resistance. The results suggest that these gene products play a crucial role in the mechanisms of plant defence against MDMV infection.

Based on the ELISA results, *Sinf* plants contained less MDMV coat protein than *inf* plants. This demonstrates the protective nature of SMM, as it may inhibit the replication and spread of MDMV in maize plants.

The results of these experiments on the effect of exogenous SMM clearly demonstrate that this natural compound has a beneficial effect on the stress response, resulting in an increase in the defence potential of maize plants during MDMV infection. These observations are in agreement with previous findings (Rácz et al., 2008; Ludmerszki et al., 2011).

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