THE PROMISING ROLE OF EXOGENOUS S-METHYL METHIONINE IN AGRICULTURE, IN THE CASE OF MAIZE CULTIVATION

Ludmerszki, E.1* – Páldi, K.1 – Rácz, I.1 – Szigeti, Z.1 – Rudnóy, Sz.1

1Eötvös Loránd University, Department of Plant Physiology and Molecular Plant Biology
H-1117, Budapest, Pázmány Péter sétány 1/C.
(phone: +36-1-381-2163; fax: +36-1-381-2164)

*Corresponding author
e-mail: ludmerszki.edit@gmail.com

(Received 30th May 2014; accepted 22nd July 2014)

Abstract. Abiotic and biotic stress conditions have negative effects on growth and yield of crop plants worldwide. The need to improve the nutritional value, stress and disease tolerance of cultivated plants is of great importance. The problem is usually solved by breeding or by the production of transgenic plants; however, the application of biologically active substances with favourable properties can be an alternative, cost- and time-effective possibility. S-methylmethionine (SMM) is an intermediate compound in the plant sulphur metabolism, being ubiquitous in the plant kingdom. SMM has important role in stress and disease tolerance of plants by taking part in plant sulphur metabolism. Previous investigations have shown that SMM has beneficial effects on the physiological status of plants and provides protection against various stress factors. The present review aims to sum up the main results concerning the beneficial effects of SMM treatment against Maize dwarf mosaic virus infection and cold stress in maize, and to reveal the possible applications of SMM in agricultural practice.

Keywords: abiotic stress, biotic stress, maize, MDMV, S-methylmethionine

Introduction

S-methylmethionine (SMM; (CH3)2-S-(CH2)2-CH(NH2)-COOH) was first detected and isolated from cabbage by McRorie et al. (1954). Later its presence has been confirmed in an increasing number of flowering plants (Sato et al., 1958; Giovanelli et al., 1980; Bourgis et al., 1999; Rácz et al., 2008). SMM is most abundant in the members of the Brassicaceae family, which plants are known for their high frost resistance (Sato et al., 1958; Greene and Davis, 1960; Giovanelli et al., 1980). SMM is a naturally occurring, biologically active non-proteinogenic free amino acid (Rácz et al., 2008). Based on previous publications, SMM was revealed as one of the most important sulphur-containing compounds in the plant metabolism, taking part in methylation processes and the regulation of methionine biosynthesis (Pimenta et al., 1998; Bourgis et al., 1999; Ranocha et al., 2000; Kočsis et al., 2003). SMM plays a vital role in the biosynthesis of osmoprotectant sulphopropionates, polyamines and prevents chlorophyll loss during stress conditions (Kočsis et al., 1998; Ko et al., 2004). SMM also promotes the incorporation of selenium into selenocysteine in selenium-tolerant plants (Neuhierl et al., 1999). Plants are characterised with sulphur autotrophy, a process in which sulphur is taken up from the nearby soil in (various) oxidised forms, than reduced and incorporated into cysteine and methionine. The latter compound can be transformed to S-adenosylmethionine (SAM), a molecule that is involved as a methyl donor in the synthesis of SMM from methionine (Kim and Leustek, 2000; Ranocha et al., 2000; Hacham et al., 2002). SMM can be reconverted to methionine, resulting in a circular
pathway, known as the SMM cycle. Based on previous results, SMM metabolism could be connected with polyamine biosynthesis (Lásztity et al., 1992; Kissimon et al., 1994; Gyetvai et al., 2002). This alternative pathway could involve the decarboxylation of SMM, resulting in an intermediate compound which can be decomposed into volatile dimethyl-sulphide and propylamine. Propylamine is the initial compound in the polyamine biosynthesis (Bagga et al., 1997). When stress factors are present first polyamine, than phenylpropanoid pathways are upregulated, resulting in production of certain phenolics, flavonoids, anthocyanins and polyamines. The utilisation of exogenous SMM increases the amount of these protective compounds, therefore it enhances the plant’s self-protection (Rácz et al., 2008; Szegő et al., 2009; Kósa et al., 2011; Ludmerszki et al., 2011; Páldi et al., 2013). Ogawa and Mitsuya (2011) determined that the intensity of the SMM cycle increased in Arabidopsis thaliana after salt treatment. Nevertheless there are no publications discussing a similar result of SMM treatment on plants, especially on maize. Researches about SMM focused on crop plants such as grape, pea and tomato. Loscos et al. (2008) measured the amino acids content present in Petit Manseng grapes and found that SMM occurs naturally and in great amounts in the plants. However, they didn’t investigate the effects of SMM on plant physiology. Tan et al. (2010) determined that SMM has positive effects on seed development in pea plants, but they didn’t use exogenous SMM treatment to further investigate the beneficial effects. Our investigations concerning maize plants therefore are novel; since we use exogenous SMM and focus on the beneficial effects against different stress factors. In this review we would like to present the major works of our team, focusing on the positive effects of SMM on maize plants against cold stress and viral infection.

Maize (Zea mays L.) is one of the most widely grown crops in Hungary, and worldwide as well. Modern maize (Zea mays ssp. mays) was domesticated in Mexico about 9,000 years ago. Nowadays, selection has focused on better yield, grain quality and agro-ecosystem adaptations (Buckler et al., 2006). Maize is mainly used as a food and feed plant, but it is getting an increasing attention as raw material for industrial and pharmaceutical products.

The protective nature of SMM against abiotic and biotic stressors in maize

Studies concerning the effects of SMM against chilling temperatures

Maize is sensitive to chilling temperatures, since it has subtropical origins, and therefore it is incapable of cold acclimation (Messing et al., 2004; Buckler et al., 2006; Kósa et al., 2009). Under 10°C injuries can be observed, among them wilting, chlorosis, necrosis are the most common, but also the reduction in the fluidity of the membranes, the loss of efficiency in the electron transport chain and a decrease in the number of membrane bound or cold-sensitive enzymes can be observed (Kocsy et al., 2004; Saibo et al., 2009). Chilling stress is often accompanied by UV-B stress causing an increased difficulty for the plant defence system. Freezing regularly occurs in late spring and can deeply influence the vegetation period of plants, altering CO₂ supplies through stomatal closure and transpiration, and affecting water uptake. Chilling tolerance is mainly related to the ability of the photosynthetic apparatus to withstand the stress caused by low temperature. Exposure to low temperature induces the synthesis of polyamines, salicylic acid and abscisic acid. UV-B radiation causes DNA damage, formation of reactive oxygen species and lipid and protein oxidation (Hannah et al., 2006; Saibo et al., 2009).
Plants evade these reactive oxygen forms by producing antioxidant enzymes, and by up-regulating the phenylpropanoid biosynthetic pathway and making secondary metabolites with sunscreen effects (such as flavonoids and hydroxycinnamic acids) (Kramer et al., 1991; Bieza and Lois, 2001; Jenkins et al., 2001).

In temperate zones, because of chilling temperature, crop yields suffer great losses in the beginning of the vegetation period (Pál and Nagy, 2002; Janda et al., 2005). Szegő et al. (2009) carried out an investigation on the protective effects of SMM treatment of maize seedlings exposed to cold stress. *Zea mays* cv. 10-day old Norma seedlings were grown for 24 h on Hoagland solution containing 0.01% SMM. After SMM treatment, seedlings were cold-stressed (5 °C for 1-24 hours) at reduced light intensity to avoid light stress. To monitor the physiological effects, the changes in the variable fluorescence (Fv/Fm) values were recorded. Fv/Fm indicates the maximum quantum efficiency of photosystem II therefore it’s a useful parameter to determine photosynthetic activity. After the cold exposure the initial 0.7 values dropped to 0.4, however, when seedlings were SMM-pretreated before cold stress, Fv/Fm values dropped only to 0.5. Results suggest that SMM pretreatment moderated the reduction in photosynthetic activity caused by cold stress. Alongside physiological measurements, Szegő et al. (2009) carried out molecular studies, where the expression of the genes involved in the polyamine biosynthesis were investigated. The greatest change was observed in the expression of arginine decarboxylase (ADC), which increased within a few hours of all the treatments, and than dropped to the level of the untreated control by the end of the 24-hour period. The increase of the gene expression was the greatest (6-7 times), when SMM treatment was followed by cold stress. In the case of ornithine decarboxylase no similar changes were observed. These data confirm previous results, showing that putrescine level rises in response to SMM (Rácz et al., 2008) and is also in good agreement with earlier observations indicating that the pathway of putrescine biosynthesis starts from arginine and is activated in response to cold stress (Rácz et al., 1996). Szegő et al. (2009) observed a similar tendency in the expression of two enzymes catalyzing the synthesis of spermidine from putrescine: SAM-decarboxylase (SAMDC) and spermidine synthase (SPDS). Based on their results, both SMM and cold treatment caused a slight increase in the gene expression; however, when the combination of the treatments was applied, higher increase was observed. The increase in the level of spermidine did not correlate with the changes in the expression level of SAMDC and SPDS, since the increase in spermidine level was greater (approximately 6 times). The data suggest that the synthesis of spermidine proceeds via a SAMDC-independent pathway; however SMM influences its expression through changing SAMDC levels and also in a direct manner (see also Lásztity et al., 1992). They also investigated the gene expression changes of CBF1, which is a regulatory protein factor of the ABA-independent pathway of stress-induced responses. The synthesis of this protein is induced by cold, drought or salt stress and is regulated by several factors. According to their results, following SMM treatment the expression of CBF1 increased to a slight extent. This rise became more pronounced after cold stress, and when the combination of the treatments was applied, the increase became even intensified. These results suggest that the rapid increase of CBF1 level aims to help the plant’s defence against cold stress, moreover this protection can be promoted with the application of exogenous SMM. Overall, Szegő et al. (2009) proved the protective nature of exogenous SMM in the course of cold stress on maize, by stimulating metabolic pathways related to the plants sulphur metabolism and polyamine biosynthesis.
Similar investigation was carried out by Kósa et al. (2011), who studied the effects of SMM and chilling temperatures on maize plants, by measuring chlorophyll a fluorescence induction and imaging, gas exchange, anthocyanin content by spectrophotometric determination and chlorophyll content. In the investigation the same cultivar of maize (Zea mays L., cv. Norma) was used. In this study, maize seedlings were grown for a longer period and the SMM treatment was applied on the 22nd day with the same concentration of SMM (0.01%). During the investigation a temperature gradient was set (6-8-10-12-14 °C) and measurements were carried out 1, 2, 3 and 4 days after chilling treatments. The chlorophyll a fluorescence parameter Fv/Fm changed similarly compared to the results of Szegő et al. (2009). When maize plants were exposed to cold stress, a decrease was observed in the Fv/Fm values, however, when SMM treatment was applied prior to the cold stress, the decrease was much lesser. The protective effect was manifested to the greatest extent during longer periods of chilling and at the lowest chilling temperatures applied. Changes in the chlorophyll content exhibited a similar tendency. The protective effect of SMM can also be attributed to the reduction of membrane damage. This statement is further validated by the photochemical quenching data derived from the chlorophyll a fluorescence, which were higher than those of non-photochemical quenching parameters when SMM treatment was applied before the chilling treatments. This indicates that the photosynthetic apparatus was able to utilise the excitation energy in a more efficient way in the electron transport chain, resulting in a better CO2 fixation. With the application of fluorescence imaging technique, an increase in the quantity of certain plant phenolic compounds was observed during combined cold and SMM treatment, as compared to the individual treatments. The results of Kósa et al. (2011) clearly demonstrates the protective effects of SMM against chilling temperatures, supplementing the results of Szegő et al. (2009).

Páldi et al. (2013) further investigated the beneficial effects of SMM treatment against cold stress by measuring the content of phenolic compounds and anthocyanins in leaves and stems, respectively, and by determining the relative gene expression of cinnamate-4-hydroxylase (C4H) and chalcone synthase (CHS). C4H and CHS are key enzymes of the phenylpropanoid pathway, leading to the synthesis of flavonoids or anthocyanins (Fraser and Chapple, 2010). Similarly, as in the case of Szegő et al. (2009) and Kósa et al. (2011), Zea mays L. cv. Norma was used as plant material. SMM treatment was carried out on 9-day-old plants, applying 0.001% SMM, and 10-day-old plants were cold stressed. During chilling temperature stress, 6 °C constant temperature was applied. Besides molecular measurements, Páldi et al. (2013) investigated the variable fluorescence parameters as well, and they acquired similar results as Szegő et al. (2009) and Kósa et al. (2011). In regard of phenolic content, chilling alone and the combined treatment of both chilling and SMM increased their amount. However, SMM did not significantly affect phenolic levels at control temperature. Based on their results it can be concluded, that SMM pretreatment enhances the response to chilling, resulting in elevated biosynthesis of some secondary metabolites. The results validate previous observations: Christie et al. (1994) demonstrated that the phenylpropanoid pathway is induced by cold stress; moreover Pourcel et al. (2007) proved that flavonoids have a direct protective role under stress conditions. Hernández and Van Breusegem (2010) stated, that flavonoids, apart from their well known protective function, may act as energy valves during abiotic stresses, which statement is further validated by the work of Páldi et al. (2013). Observing the transcript levels of C4H, they found that the effect of SMM alone was slight; on the other hand, cold exposure raised the transcript level
significantly. This increase was more pronounced when SMM treatment was applied prior to cold stress. Changes in the expression of CHS were similar to C4H, with smaller amplitude but with the same tendency. According to their results, C4H expression was increased to a greater extent by cold stress than CHS. Since C4H is an earlier step in the phenylpropanoid pathway, this indicates a general activation of the pathway either by SMM treatment or cold stress. As concluded, SMM pretreatment stimulates the phenylpropanoid pathway and helps the plant to avoid cold injuries. The results are in great correlation with Szegő et al. (2009) and Kósa et al. (2011).

**The protective nature of SMM treatment against Maize dwarf mosaic virus infection**

Apart from abiotic stressors, investigations were carried out to study the beneficial effects of SMM against biotic stressors as well, namely in the case of Maize dwarf mosaic virus (MDMV) (Ludmerszki et al., 2011). The virus spreads in natural or in agro-environments, via aphids, seed or pollen transmission (Astier et al., 2007; Tóbiás et al., 2008). The symptoms of the infection are reduced growth and chlorotic bands on the leaves, giving a mosaic pattern. The infection usually causes 10-45% crop losses; however, the damage can reach 100% (Tóbiás et al., 2008). Ludmerszki et al. (2011) used a different subspecies of maize (Zea mays cv. saccharata Koern, sweet corn), which was susceptible to the infection. MDMV infection was carried out one day after the SMM treatment on 11-day old plants, and the infection was repeated two days after the first one to ensure successful infection. MDMV Dallas-A strain was used for the infection. In infected plants the chlorophyll content decreased. At the same time, an increase in the 520 nm of fluorescence was observed, indicating that MDMV triggers molecular pathways involved in the synthesis of protective compounds with a fluorescence peak of 520 nm (see also Pineda et al., 2008). SMM pretreatment resulted in the protection of chlorophylls and an enhancement of fluorescence at 440 nm, indicating an increase in the concentration of compounds with a fluorescence peak at 440 nm. Based on the ELISA results, SMM pretreatment decreased the amount of viral coat proteins present in infected leaves. A close look at the data indicates that SMM may inhibit the replication and/or the spread of MDMV.

Uzarowska et al. (2009) previously showed that in infected maize plants the expression levels of S-adenosylmethionine synthase (SAMS) and a 14-3-3-like protein GF14-6 significantly increased, probably due to the complex biochemical pathways of plant defence. Our results support these data, showing that SAMS and GF14-6 both play a crucial role in the plant defence mechanism against MDMV infection. SMM and MDMV individually trigger pathways that are related to high levels of GF14-6 expression, however, when the combination of the treatments was applied, an inhibition of GF14-6 expression was observed. SMM pretreatment delayed the increase of SAMS expression, but also prolonged it, providing a slow, but constant rise. Results of Ludmerszki et al. (2011) demonstrated that the natural compound SMM has beneficial effects on biotic stress response reactions, resulting in an increase in the defence potential of maize plants during MDMV infection.

**Possible applications of SMM in agricultural practice**

Based on the investigations listed above it is clearly demonstrated that in maize SMM treatment provides protection against chilling temperatures and MDMV infection. SMM was applied to at least 9-days old plants in the investigations, but previous studies
indicated, that germination of the seeds in SMM-containing solutions may also provide protection against environmental factors (unpublished data). Our team is still carrying out investigations concerning SMM treatment during germination. In the 1980’s when Kékfrankos grape was sprayed with SMM wine quality, anthocyanin content, free acid content and crop mass increased (unpublished data). On tomato SMM induced the synthesis of certain polyamines, being important in plant defence. Our ongoing studies indicate that SMM pretreatment could also be successfully applied in the case of drought and salt stress. All of these results lead to the conclusion, that the application of SMM can achieve a success in the field of agriculture, by moderating harmful effects of certain stress factors.

Acknowledgements. This work was supported by the European Union and the State of Hungary, co-financed by the European Social Fund in the framework of TAMOP 4.2.4. A/1-11-1-2012-0001 ‘National Excellence Program’, and by the grant of the Hungarian Scientific Research Fund (OTKA 73178).

REFERENCES


[34] Páldi, K., Rácz, I., Szigeti, Z., Rudnóy, S. (2013): S-methylmethionine alleviates the cold stress by protection of the photosynthetic apparatus and stimulation of the phenylpropanoid pathway. – Biologia Plantarum in press.


