Gene expression of glutathione-S-transferase in sunflower (*Helianthus annuus* L.) inoculated with arbuscular mycorrhizal fungi under temperature stresses

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Abstract: The association between terrestrial plants and arbuscular mycorrhizal (AM) fungi is one of the most widespread mutualistic plant-fungal interactions in natural and agricultural systems. Several studies suggest that AM symbiosis can help plants to alleviate various biotic and abiotic stresses. Some works show clearly that the amelioration of stress resistance by AM symbiosis is often related to the enhancement of antioxidant levels or activities in mycorrhizal plants. Glutathione-S-transferase is playing an important role in the protection against oxidative membrane damage and necrotic disease symptoms. Plants of different age, inoculated and non-inoculated with arbuscular mycorrhizal fungi were exposed to high temperature- and low temperature stresses. To detect the expression profiles of glutathione-S-transferase gene, total RNA was extracted from sunflower leaves 24hrs after the stress exposition and quantitative real-time PCR was carried out using modified primers. The expression of glutathione S-transferase gene changed under different temperatures influenced by the age of the plants. The highest level of glutathione-S-transferase gene expression was measured in mycorrhizal plants during high temperature stress compared to control plants.
Keywords: plant, mycorrhiza, stress, gene expression

Introduction

The association between terrestrial plants and arbuscular mycorrhizal (AM) fungi is one of the most widespread mutualistic plant-fungal interaction in natural and agricultural systems. This symbiotic relationship evolved more than 400 million years ago facilitating the colonization of land by plants (Taylor et al. 1995; Smith and Read 1997; Redecker et al. 2000; Smith and Read 2008).

Some biotic and abiotic stress conditions generate reactive oxygen species (ROS) in plant tissues causing damage to proteins, lipids and photosynthetic pigments as well. By ROS-scavenging enzymes (catalase, superoxid dismutase and peroxidase) the plants can detoxify the oxidative ROS (Mittler 2002; Passardi et al. 2004; Matsumura et al. 2007; Kohler et al. 2009). Glutathione S-transferase is considered as an antioxidative enzyme, it plays an important protection role in the plant defense system against oxidative stress, oxidative membrane damage and necrotic disease symptoms (Marrs 1996; Sharma et al. 2004).

The regulation of host antioxidant compounds by AM and the related mechanisms are not well known, and only a few investigations have been carried out at different stages of mycorrhizal colonization. However, an in-depth investigation could help to elucidate the mechanisms of tolerance induced by AM symbiosis and to discriminate the stress-induced processes. Therefore, the main objectives of current research were to estimate changes of the expression of glutathione S-transferase in plant-arbuscular mycorrhizal system under temperatures stresses for evaluating AMF as biocontrol at different stages of mycorrhizal colonization.

Materials and methods

Seeds of sunflower (*Helianthus annuus* L.) were germinated and the four-day-old pre-

germinated seeds were sown to plastic pots containing substrate (soil:sand 1:1, v/v), previously sterilized in autoclave for 25 minutes at 121°C, 1.2 bar. The seedlings were grown in growth chamber under controlled condition with a 16 hour day length and at a temperature of 24 °C ± 0.5 °C. The moisture of substrate was 60 %.

The commercial product Symbivit® (mixture of *Rhizophagus irregularis* BEG140, *Funneliformis mosseae* BEG95, *Claroideoglomus etunicatum* BEG92, *Claroideoglomus claroideum* BEG96, *G. microaggregatum* BEG56, *Funneliformis geosporum* BEG199) produced by Symbiom Ltd. (Lanskroun, Czech Republic; www.symbiom.cz) was applied at 15 g of inoculum (consisting of 80 propagules g^{-1}) per seedling into the planting hole and seedlings were planted immediately.

Healthy 15 and 42 days-old plants (mycorrhizal- and non-mycorrhizal plants) were selected and exposed to two temperature stress conditions. Plants were exposed to 38 °C for 24 h. (High temperature stress, HT) or were incubated for 24 h at 4 °C (Low Temperature stress, chilling stress LT). After 24 h the leaves were collected and analyzed. All treatments replicated in 5 times: non-mycorrhizal and mycorrhizal plants, 15 days old and 42 days old plants and low and high temperature stresses, altogether 40 plants were selected for measurements.

qRT-PCR

Total RNA was extracted from 15 and 42 days-old sunflower leaves using Vantage Total RNA Purification Kit (Origene, USA) according to the manufacturer's instructions, followed by DNase I (Fermentas) digestion to remove residual genomic DNA contamination. For qRT-PCR analysis, first-strand cDNA was synthesized from varieties of all treatments using First-strand cDNA Synthesis for Quantitative RT-PCR kit (Origene, USA).

Modified glutathione S-transferase oligonucleotide primers
GST-f (5'-GAGAAGGCTCAGGCTCGATT-3') and GST-r
(5'-GCAACAGCTTGCTTCTCTCC-3') (Radwan et al. 2005) were used to amplify the glutathione S-transferase gene from sunflower.

PCR cycling program for GST and actin genes consisted of 15 min at 95°C, followed by 40 cycles of 15 sec at 95°C, 30 sec at 57°C and 16 sec at 72°C, with an additional cycle (60 sec at 95°C, 30 sec at 57°C and 30 sec at 95°C) in the end. The real-time PCR experiment was carried out at least three times under identical conditions. The real-time PCR efficiency was determined for each gene (actin gene was used as an internal reference, GST) and each stress condition using the $2^{-\Delta\Delta CT}$ method. QRT-PCR was performed on Mx3000P QPCR System (Agilent). The root colonization (internal fungal structures: hypae, arbuscules, vesicules) were examined using the gridline intersection method (Giovanetti and Mosse 1980).

Results and discussion

In the earlier stage (15 days old plants) the high temperature stress caused more intensive gene expression of glutathion S-tranferase in the measurements of the earlier stages (15 days old) of inoculated plants compared to the control (Fig.1.). Lower levels of gene expression were measured in the plants exposed to the low temperature as for the high temperature stress GST expression was higher in plants inoculated compared to those not inoculated.

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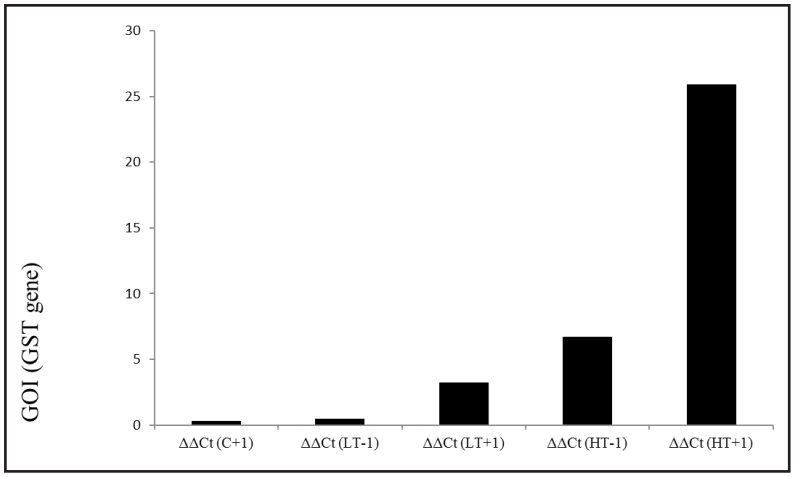

Figure 1. Glutathione S-transferase gene expression in 15 days old plants

y axis: **GOI**, Gene of Interest (relative amounts of the examined gene (GST) compared to the control sample (C− = Ø)), *x axis:* **C+**, Control, inoculated with mycorrhizal fungi, **LT−**, Low Temperature stress, non-inoculated with mycorrhizal fungi, **LT+**, Low Temperature stress, inoculated with mycorrhizal fungi, **HT−**, High Temperature stress, non-inoculated with mycorrhizal fungi, **HT+**, High Temperature stress, inoculated with mycorrhizal fungi

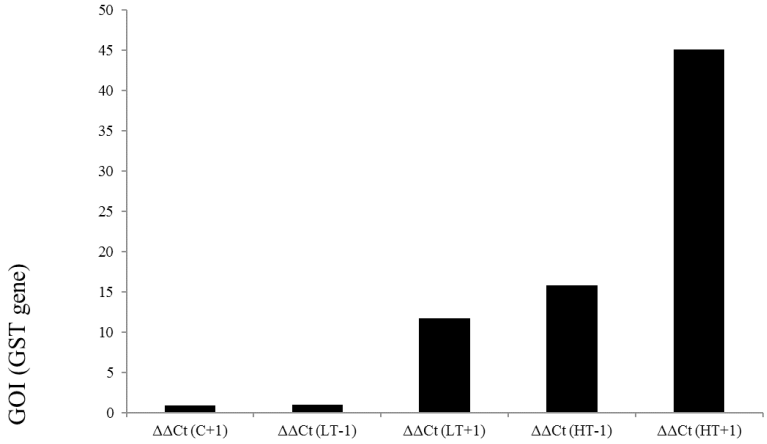

Figure 2. Glutathione S-transferase gene expression in 42 days old plants

y axis: **GOI**, Gene of Interest (relative amounts of the examined gene (GST) compared to the control sample (C− = Ø)), *x axis:* **C+**, Control, inoculated with mycorrhizal fungi, **LT−**, Low Temperature stress, non-inoculated with mycorrhizal fungi, **LT+**, Low Temperature stress, inoculated with mycorrhizal fungi, **HT−**, High Temperature stress, non-inoculated with mycorrhizal fungi, **HT+**, High Temperature stress, inoculated with mycorrhizal fungi

In the measurements of the older (42 days old) plants compared to the 15 days old plants have clearly showed similar trend of gene expression (Fig.2.). Under high temperature stress conditions the inoculated ones showed especially higher levels of gene expression than the non-inoculated ones. Likewise under low temperature stress conditions the inoculated ones, which had a massive mycorrhizal fungi colonization (inoculation of the plants by arbuscular mycorrhizal fungi were higher than 70%) had showed a higher level of gene expression than the non-inoculated plants and control ones.

Conclusions

Glutathione S-transferase belongs to the most studied enzyme groups and there are several studies about their function in the cell defend system, like inactivation of the reactive oxygen species (Edwards et al. 2000). Our results demonstrate that mycorrhizal inoculation at different stages stimulates abiotic stress responses in the plants. On the basis of these data we provided a deeper insight into the role of arbuscular mycorrhizal fungi in arresting reactive oxygen species and strengthening antioxidant defense system in the host plants. However, although our results could help the better understanding of various stresses resistance mechanisms, more additional experiments are required to clarify the molecular basis underlying the regulation processes of the enzymes involved. In the future, we plan to extend the experiment to other genes as well. The length of the proceeding book limits the deeper explanation of this work.

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