When the cultured cells of Glycine max (soybean) were treated with 5 mM geraniol as a chemical stress, an mRNA level was elevated in a rapid but transient increase. The mRNA was cloned and sequenced, and found to correspond to the mRNA encoding glutathione S-transferase (GST). The GST mRNA level and GST activity were elevated to maxima at 4–6 h and 8 h, respectively, after treatment of the cultures with geraniol. These indicate that GST is one of the geraniol-responsive factors in soybean cells.

Key words: Glycine max; geraniol; glutathione S-transferase; chemical stress; defense reaction

In our recent studies on the defense reaction in plant cells against chemical stress, we have shown that geraniol was a potent inducer of apoptosis-like cell death in plant cells. On the other hand, we purified and characterized a 20-kDa protein (p20) with trypsin inhibitory and GTP-binding activities from the cultured cells of Glycine max (soybean), and have also cloned and characterized the p20 cDNA from soybeans. In the investigation of a function of p20 in the signal transduction of plant cells against chemical stress, we have now found that glutathione S-transferase (GST) is a kind of geraniol-responsive factor in the cultured soybean cells.

In order to examine the effects of geraniol on mRNA expression during the defense reaction of plant cells against chemical stress, the p20 mRNA contents of cultured cells following geraniol treatment were measured on the basis of the reverse transcriptase-polymerase chain reaction (RT-PCR). For the treatment experiment, geraniol was added to suspension soybean cells to a concentration of 5 mM and the cultures were incubated at 25°C. At regular intervals, part (0.5 g) of the cultures was filtered out and powdered in liquid nitrogen. Total RNA was isolated from the powder with the guanidium thiocyanate-phenol-chloroform extraction method. The adapter primer (Takara RNA PCR kit) with M-MLV reverse transcriptase (Sawaday Technology Co., Ltd.) was used to start the synthesis of soybean single-stranded cDNA from the total RNA. A PCR primer GSTI-1 (5′-GTG GCA CAT ACT ATG TGT TGC CAG-3′) was constructed from the p20 clone. The single-stranded cDNA was used as a template for amplification with EX Tag DNA polymerase (Takara Shuzo Co., Ltd.), the upstream primer (GSTI-1) and the downstream primer (oligonucleotide derived adaptor primer). The amplification was done at 94°C for 1 min for denaturation, 64°C for 1 min for annealing, and 72°C for 1 min for synthesis, followed by 30 cycles. The PCR products were electrophoresed on an agarose gel and measured by densitometry of the ethidium bromide-stained gel.

Amount of two RT-PCR products of about 300 base pairs (bp) and 400-bp were found to be changed by the geraniol treatment. The amount of 400-bp fragment was expressed stably for 6 h after treatment and then decreased gradually. On the other hand, a 300-bp fragment was found to increase rapidly but transiently, before a decrease of the 400-bp fragment. The 400-bp fragment was identified as p20 mRNA by sequencing. The 300-bp PCR product was excised from the agarose gel, purified, and ligated to the pPCR-Script SK(-) vector (Stratagene). DNA sequence analysis was done with an ABI PRISM310 Genetic Analyzer with a BigDye terminator cycle sequencing kit (PE Biosystems). This nucleotide sequence (Accession Number AB039373) was identical with the soybean glutathione S-transferase, except for two nucleotide sequences in the 3′-untranslated region. The deduced amino acid sequence was completely identical with the GST. This result revealed that the amplified fragment induced by geraniol is the GST. The clone is designated GmGST (Glycine max glutathione S-transferase). The nucleotide sequence of a segment of the GmGST cDNA was completely identical to the 10-mer sequence of 3′-end in GSTI-1 primer. Accordingly, it is conceivable that GmGST can be isolated with a primer constructed from the p20 cDNA.

Therefore, we next examined the course of the
Changes in the GST activity of the cell-free extract.

As shown in Fig. 1A, when cultured cells of soybean were treated with geraniol, the GmGST mRNA level began to increase after 1 h, reached a maximum of 7-fold over the untreated level after 4–6 h, and then decreased gradually after 8 h. This change in the GmGST mRNA content preceded the increase in GST activity, which began after 4 h and was maximal after 8 h, as shown in Fig. 1B. These results strongly suggest that, in the cultured cell system, the initial increase in GST is transcriptionally regulated through the level of GmGST mRNA.

Recently, McGonigle et al. reported that 25 soybean GSTs are divided according to sequence similarity into three types, I (4), II (1), and III (20). The geraniol-responsive GmGST was identical with the GmGST2, belonging to type III. The GmGST2 had the highest homology with the GmGST10 in GmGST family. To investigate whether geraniol-inducible GmGST induction is specific to GmGST2, the GmGST10 mRNA expression was measured by use of two primers (5'-TTA CTT GAA CAT TCC CTA TGA-3' and 5'-ACA TGA AAC CTA CTC GAT GAC -3') derived from GmGST10 sequences that same position of GmGST2. The primers derived from GmGST2 had little similarity with all of the GmGST family except GmGST2. When cultured cells of soybean were treated with or without geraniol for 4 h and 6 h, the GmGST10 mRNA expression was found to be less than 5% compared with GmGST2 mRNA expression in all points. This result suggests that the induction of GmGST2 by the chemical stress with geraniol is specific.

The soybean GST promoter was previously reported to be activated by a wide range of chemical agents. In the carnation plant a DNA-binding protein interacts with an ethylene-responsive enhancer element of the GST promoter. Promoter analyses of GSTs regulated by auxin are reported in tobacco and Arabidopsis. It was well known that glutathione plays a pivotal role in protecting plants from environmental stresses, oxidative stress, xenobiotics, and some heavy metals. Plants detoxify many organic contaminants by conjugating them to glutathione for storage or further metabolism. These reactions are catalyzed by GSTs and they also have it glutathione peroxidase activity and ligand-binding functions. The GmGST may have functional importance for the defense reactions of plant cells to chemical stress. Further study is necessary to clarify the function of the GmGST in the plant cells.
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