Characterization and expression of cold-induced glutathione S-transferase in freezing tolerant Solanum commersonii, sensitive S. tuberosum and their interspecific somatic hybrids

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Received 3 May 1999; received in revised form 17 November 1999; accepted 30 November 1999

Abstract

Glutathione S-transferases (GST) form a large family of non-photosynthetic enzymes known to function in detoxification of xenobiotics. We have cloned and characterized a novel, low temperature regulated GST, Solanum commersonii glutathione S-transferase (Sgst1), from a cold acclimated wild potato species S. commersonii and studied the level of its transcription in freezing tolerant and sensitive Solanum genotypes. Active oxygen species (AOS) were associated with the early steps of Sgst1 regulation since a strong mRNA signal was detected in hydrogen peroxide and salicylic acid treated plants. In experimental conditions where the formation of AOS is known to accelerate, such as excessive light at low temperature, significant accumulation of the transcript was observed in S. commersonii. Under similar experimental conditions, Sgst1 transcript did not accumulate in freezing sensitive S. tuberosum eventhough a single copy of the Sgst1 sequence was present in both species. Thus, Sgst1 in the S. tuberosum genome did not exhibit the same cold-induction properties as in S. commersonii. In comparison with the parental lines, the somatic hybrid SH9A (S. commersonii (+) S. tuberosum) had an interparental level of Sgst1 accumulation as well as freezing tolerance. The abundance of Sgst1 transcript thus correlated well with the freezing tolerance of the parental lines and the somatic hybrid SH9A. Increased GST enzyme activity was observed in S. commersonii and SH9A after 2 days of cold acclimation whereas the activity declined in S. tuberosum during the same period. Further studies of potato lines (S1) that were derived by selfing the somatic hybrid revealed a more complex relationship between freezing tolerance and Sgst1 expression level. In the S1 genotypes, the regulation of Sgst1 transcription resembled more that of S. tuberosum and was not directly related to their freezing tolerance. This could be due to the interaction of the two genomes in S1 genotypes as well as chromosomal rearrangements during meiosis. © 2000 Elsevier Science Ireland Ltd. All rights reserved.

Keywords: Cold acclimation; Gene expression; Freezing tolerance

1. Introduction

Glutathione S-transferase (GST) catalyses the conjugation of glutathione to several electrophilic substrates and, therefore, it is a part of the complex detoxification system of xenobiotics and oxygen radicals in plant and animal cells [1]. The detoxification system has three roles: recognition, compartmentalization and metabolism of cytotoxic compounds. The entire detoxification system includes cytochrome P450 monooxidase, which oxidizes the toxic compounds, and GSTs and glucosyltransferases that conjugate the toxic molecules to glutathione (GSH) or sugars. The toxic molecule–GSH conjugate can then be transported to the vacuole or apoplast and metabolized to a non-toxic compound. Based on the substrate specificity, GSTs are divided in five subclasses; α, μ, π, σ and θ [1,2]. All currently identified plant GSTs belong to the most ancient forms of GSTs,
to the 0 subclass. The 0 GSTs have evolved in anaerobic bacteria for their ability to prevent oxygen toxicity [2]. In plants, the theta-GSTs have been further classified in type I, II and III according to their amino acid sequence and positions and number of exon/intron sequences [3].

The detoxifying activity of GSTs has been found to be associated with pathogen attack, oxidative and heavy-metal stress, as well as in regular metabolic processes such as auxin response and metabolism of secondary products [2]. In plants, the first known function of GSTs was related to the detoxification of atrazine herbicide [2]. Several researchers have reported the function of GSTs in response to auxins (parB, [4]; Hmgst1, [5]) and pathogen attack (Gmhsp-26A, [6]). In maize, type I GST, Bz-2, is actually the last known step in the anthocyanin biosynthetic pathway [7]. Soybean GST, GmGST26A, sharing low sequence homology with maize Bz:2 has also been found to function in anthocyanin biosynthesis [8]. In potato, one of the pathogenesis-related proteins, prp1-1, was characterized as a pathogen-inducible gene with multiple copies [9] and later on identified as GST (Accession J03679).

Many GSTs are multifunctional and conjugate a wide variety of substrates that the enzyme conjugates to glutathione. Some GSTs have a substrate preference to hydroperoxidase, the toxic byproduct of lipid peroxidation in plants [2]. Several environmental stresses, freezing stress among them, can damage cell membranes and increase lipid peroxidation. Consequently, it has been postulated that membranes are the primary site for freezing injury in plants [10]. The importance of controlling lipid peroxidation in membranes during chilling stress has been supported by results using transgenic plants where the over-expression of GST with glutathione peroxidation activity in tobacco, improved the growth rate of the transgenic seedlings at chilling temperatures [11].

The improvement of freezing tolerance during cold acclimation requires alterations in gene expression and protein metabolism [12]. Among Solanum species the effect of acclimation on gene expression has been studied in two wild potato species: Solanum commersonii [13,14] and in Solanum sagraandinum [15]. In S. commersonii, two osmotin-like genes have been identified that were not only low temperature, but also ABA and pathogen inducible [13]. Several dehydrin-genes are known to be induced during exposure to environmental stresses such as low temperature and drought [16]. The presence of similar dehydrin sequences has been observed in both freezing-sensitive Solanum tuberosum and -tolerant S. commersonii [14]. The expression of the dehydrin-gene was similarly induced in both species during cold acclimation. In S. sagraandinum the differences in the induction and stability of twelve cold induced genes were observed during cold acclimation [15]. Sequence homology revealed that part of these low temperature regulated genes were probably responsible for the protection of cellular and chloroplastic functions during stress; whereas some of them were related to metabolic adjustment to low temperature [15,17].

Cultivated S. tuberosum varieties suffer from serious freezing damages if the temperature drops below – 3°C for just a few hours. In contrast, S. commersonii can tolerate lower temperatures (–4.6°C) [18]. As a first step in improving the freezing tolerance of cultivated potato varieties, we have previously characterized the freezing tolerance and acclimation capacity of a potato progeny (S1) derived by selling a somatic hybrid between S. commersonii and S. tuberosum (SH9A) [18,19]. The objective of the present investigation was to study the molecular basis of freezing tolerance of S. commersonii as compared to S. tuberosum and selected genotypes of the S1 progeny. We have isolated and characterized a low temperature regulated glutathione S-transferase (Scgst1) cDNA from cold acclimated S. commersonii and studied the gene expression in relation to freezing tolerance. The possible function of the Scgst1 gene product is discussed.

2. Materials and methods

2.1. Plant material and cold acclimation treatments

The dihaploid Solanum tuberosum clone SVP11 and the S. commersonii accession PI243503 were used to produce interspecific somatic hybrids [19]. The fertile somatic hybrid (SH9A) was selfed and the progeny (S1) was tested for non-acclimated and cold acclimated freezing tolerance [18]. Six genotypes were selected and characterized as tolerant (S. commersonii, 1020, 2019), intermediate
(SH9A) or sensitive (S. tuberosum (SPV11), 2051) to freezing. Plants were propagated from stem cuttings and grown for 4–6 weeks under greenhouse conditions at 20/15°C day/night with an 18-h photoperiod of 220 μmol m⁻² s⁻¹ supplied by SON-H/350 W lamps (Philips, Belgium). Subsequently, plants were cold acclimated in a growth chamber either at 4/2°C or at 2/1°C (night/day) in a 14-h photoperiod of 80 μmol m⁻² s⁻¹ for 1–7 days. Light intensities of 50–80 μmol m⁻² s⁻¹ were used for low light treatments and 700 μmol m⁻² s⁻¹ for high ones. Simultaneously temperature was gradually increased (2°C h⁻¹) from 10°C either to non-freezing (2°C) or freezing (−3°C) night frost. Samples were collected of plants kept at the frost temperature for 1 h.

2.2. Plant growth regulators, salicylic acid, hydrogen peroxide, drought and heat treatments

Solanum commersonii plants were propagated in vitro in Murashige–Skoog (MS) medium solidified with 7 g 1⁻¹ agar and grown for 3 weeks. The plants were then submerged to fresh MS-solution supplemented with 1 μM IAA, 1 μM 2,4-D, 1 mM SA or 0.1 mM H₂O₂ for 4 h at room temperature; thereafter the samples were harvested. For the drought treatment, plants were air-dried for 1 h at room temperature. Heat-stressed plants were subjected to a temperature program that increased the temperature gradually 4°C h⁻¹ to 40°C and maintained at the maximum temperature for 1 h [13].

2.3. Isolation and sequencing of Scgst1

Total RNA was extracted from 3-, 5- and 7-day cold acclimated (2/1°C) leaf material of Solanum commersonii [20]. The amount of extracted RNA was quantified by spectrophotometer and equal amounts of total RNA from each cold acclimation treatment were mixed and used for the preparation of the cDNA library. Poly(A)⁺ was isolated from total RNA by means of oligo-dT-cellulose column (Pharmacia). Complementary DNA synthesis and construction of the cDNA library was carried out according to the instructions provided by the supplier (UniZap-cDNA Synthesis Kit, Stratagene, La Jolla, CA). Approximately 3 × 10⁵ phages were differentially screened using replica plates and αP³² labeled single-stranded cDNA probes prepared from non-acclimated and acclimated poly(A)⁺ RNAs. cDNAs corresponding to cold induced mRNA were isolated by three steps of differential screening and the positive recombinant cDNAs were in vitro excised into pBluescript SK- following the supplier’s (Stratagene) specifications. The expression of cold-specific cDNAs was verified by northern hybridization using cDNA inserts as probes. Both strands of the acclimation-specific cDNA clone were automatically sequenced (Center for Gene Research and Biotechnology, Oregon State University, Corvallis, OR 97331, USA) and identified by comparison with the EMBL gene database. The isolated S. commersonii glutathione S-transferase (Scgst1) sequence will appear in the EMBL and GenBank™ Sequence Nucleotide Databases under the accession number AF002692.

2.4. Nucleic acid isolation and analysis

For Northern hybridization, total RNA was isolated by the Plant RNeasy extraction kit (QIA-GEN) and 10 μg was size-fractionated on 1.5% (w/v) agarose formaldehyde gels. RNA was blotted onto nylon transfer membranes (MagnaGraph, Microseparations) essentially as described by Sambrook et al. [21] and prehybridized for 1 h at 42°C in hybridization solution (5xSSPE, 0.5% SDS, 5xDenhardt’s, 25% formamide, 100 μg ml⁻¹ ss-DNA). The 981 bp long Scgst1 isolated from S. commersonii was used as a probe. The purified cDNA probe was ³²P-labeled by nick translation (Pharamacia) and used for the hybridization. Membranes were washed once with 2xSSC, 0.1% SDS at 65°C for 5 min; once with 1xSSC at 65°C for 20 min and finally once with 0.2xSSC, 0.1% SDS at 65°C for 10 min. After visualization of the corresponding mRNA by autoradiography, the probe was removed by stripping the membranes according to the instructions provided by the supplier. Equal RNA loading was verified by rehybridization of the membranes with a ³²P-labelled probe corresponding to a soybean ribosomal gene, which was kindly provided by Gary Coleman, College Park, MD, USA.

2.5. Southern analysis

For Southern analysis, genomic DNA was isolated [22] and 10 μg of total DNA was digested with EcoRI, HindIII and XhoI. The DNA gel blot
analysis followed general procedures [21]. Hybridizations were performed as described for the northern hybridizations.

### 2.6. Glutathione S-transferase enzyme activity

Total glutathione S-transferase (GST) activity was assayed as described by Marrs and Walbot [23] with some modifications. Briefly, 1 g of leaf material was homogenized in 2 ml of 0.1 M sodium phosphate buffer (pH 6.5) and filtered through Sephadex G-25 Medium columns. Protein extracts (50 µg total protein) were combined with 10 mM GSH (Sigma) and 3 mM 1-chloro-2,4-dinitrobenzene (CDNB) (Sigma) in 0.1 M sodium phosphate buffer (pH 6.5). The rate of change in $A_{340}(A_{340} \times 10^{-3} \text{ min}) \mu g^{-1} \text{ protein}^{-1}$ was measured by spectrophotometer (Shimadzu UV-160A). Background levels of spontaneous CDNB conjugation were subtracted. Two independent experiments with two replications were conducted.

### 3. Results

#### 3.1. Isolation of Scgst1 and sequence comparison to known glutathione S-transferases

Both strands of the isolated cDNA insert were sequenced. The putative amino acid sequence revealed a 52 bp untranslated leader sequence, followed by an open reading frame coding for a 213 amino acid polypeptide and 3’ untranslated region of app. 300 bp. The comparisons of the nucleotide sequence and the predicted amino acid sequence with other known sequences were made by using BLAST 2.0 program and the latest versions of the GenBank™ (updated April 1999). The search revealed that the amino acid sequence has 88% nucleotide identity to an auxin binding protein of Hyoscyamus muticus (accession X78203), which was characterized as type III glutathione S-transferase (GST). However, significant similarities were observed with other members of type III GSTs, including aluminum and phosphorous starvation inducible GST from Nicotiana tabacum (71%) (accession D29680) and another auxin inducible gene, parB, isolated from Nicotiana tabacum (69%) (accession D10524). The cDNA sequence isolated in the present study was named Scgst1 referring to Solanum commersonii glutathione S-transferase. No significant sequence homology was seen with GST, prp-1, isolated from potato (accession J03679). A restriction map revealed one EcoRI site in the 3’ end of Scgst1 cDNA (data not shown). DNA hybridization on EcoRI, HindIII and XhoI digested total DNA revealed the presence of one copy of Scgst1 in the S. commersonii genome (Fig. 1). A single copy of Scgst1 was also present in S. tuberosum clone SPV11 and in the somatic hybrid SH9A. There were no differences in the hybridization patterns between the genotypes studied indicating that the general organization of the gene was similar.

![Fig. 1. Southern blot analysis of Scgst1 gene in Solanum commersonii (scmm), S. commersonii (+) S. tuberosum somatic hybrid (SH9A) and dihaploid S. tuberosum clone (SPV11). Total DNA (10 µg) was digested with EcoRI, HindIII and XhoI. DNA size markers at the right are indicated in kb.](image-url)
3.2. Scgst1 transcription and glutathione S-transferase enzyme activity at low temperature

Scgst1 mRNA accumulated significantly by cold acclimation at 2/1°C for 2 days (Fig. 2). Two transcripts, app. 1.1 and 1.0 kb in size, were detected by RNA hybridization whereas only the smaller transcript (1.0 kb) (indicated by an arrow) accumulated at low temperature. Elevated level of Scgst1 transcript was observed also after 7 days of cold acclimation (Fig. 3). Cold acclimation increased the amount of Scgst1 mRNA in S. commersonii slightly more than in S. tuberosum (Figs. 2 and 3). Freezing tolerance was improved by 2-day cold acclimation S. commersonii by 1.5°C whereas in S. tuberosum significant changes were not observed (Table 1).

Total glutathione S-transferase activity (GST) was measured from 0 to 2 days cold acclimated plants. In S. commersonii and somatic hybrid SH9A, increased GST activity was measured after 2 days of cold acclimation (Table 2). GST activity was highest in non-acclimated S. tuberosum. In contrast to S. commersonii and SH9A, GST activity declined in S. tuberosum during cold acclimation (Table 2).

3.3. Induction of Scgst1 transcription in S. commersonii by plant growth regulators and environmental stresses

A remarkable accumulation of Scgst1 transcript was detected after 4-h incubation of in vitro-plants of S. commersonii in 1 mM salicylic acid or in 0.1 mM H2O2 solution (Fig. 4). Heat treatment at 40°C also resulted in significant accumulation of the transcript whereas exposure to drought stress had smaller effect. Plant growth regulators such as IAA or 2,4-D did not alter Scgst1 transcription significantly (Fig. 4) nor did a 4-h incubation in either 100 μM ABA or 100 mM NaCl (data not shown). Similar results were obtained when detached leaves of in vivo plants were submerged to the same solutions as the in vitro plants for 1 or 4 h (data not shown).

Table 1

<table>
<thead>
<tr>
<th>Genotype</th>
<th>LT50 (°C)</th>
<th>1CA</th>
<th>2CA</th>
</tr>
</thead>
<tbody>
<tr>
<td>NA</td>
<td>-4.8 ± 0.2</td>
<td>-5.8 ± 0.1</td>
<td>-6.4 ± 0.5</td>
</tr>
<tr>
<td>S. commersonii</td>
<td>-3.8 ± 0.2</td>
<td>-4.3 ± 0.4</td>
<td>-5.2 ± 1.1</td>
</tr>
<tr>
<td>somatic hybrid,</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SH9A</td>
<td>-3.2 ± 0.6</td>
<td>-3.6 ± 0.1</td>
<td>-3.9 ± 0.8</td>
</tr>
<tr>
<td>S. tuberosum</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>cv. SPV11 1020</td>
<td>-4.4 ± 0.5b</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2019</td>
<td>-4.3 ± 0.2b</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2051</td>
<td>-3.1 ± 0.3b</td>
<td></td>
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a See Section 2 for detailed description of the plant material. S.E. of the mean, n = 3.
b LT50 values obtained from Seppänen et al. [18].

Fig. 3. Accumulation in S. commersonii scmm, the somatic hybrid SH9A and S. tuberosum SPV11 of Scgst1 mRNA without (na) and with cold acclimation (ca) at 4/2°C (day/night) for 7 days. RNA blots were hybridized as described in Fig. 2 and in Section 2.

Fig. 2. Induction of Scgst1 transcription during the first 2 days of cold acclimation at 2/1°C in Solanum commersonii scmm, the somatic hybrid SH9A and the dihaploid S. tuberosum clone SPV11. RNA (10 μg per lane) was hybridized with 32P-labelled Scgst1 cDNA or soyabean ribosomal gene (rRNA) as described in Section 2.
Table 2
Total glutathione S-transferase (GST) activity (ΔA₄₅₀ min⁻¹ µg⁻¹ protein) in non-acclimated (NA) and 1–2 days cold acclimated (1CA, 2CA) potato plants

<table>
<thead>
<tr>
<th>Genotype</th>
<th>GST activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NA</td>
</tr>
<tr>
<td><em>S. commersonii</em></td>
<td>0.32 ± 0.08</td>
</tr>
<tr>
<td>SH9A</td>
<td>0.10 ± 0.04</td>
</tr>
<tr>
<td>SPV11</td>
<td>0.70 ± 0.28</td>
</tr>
</tbody>
</table>

*a S.E. of the mean, n = 4.

Fig. 4. Induction of Scgst1 gene expression in *S. commersonii*. Plants were incubated for 4 h in MS solution (control) or in MS solution containing 1 µM IAA, 1 µM 2,4-D, 1 mM SA or 0.1 mM H₂O₂ or exposed to heat (H) or drought (D) treatments. RNA blots were hybridized as described in Fig. 2 and in Section 2.

3.4. Induction of Scgst1 by light or low temperature

To further study the early steps of Scgst1 induction in response to low temperature stress, non-freezing (2°C) and freezing (−3°C) night frosts combined with low (LL) (80 µmol m⁻² s⁻¹) or high (HL) (700 µmol m⁻² s⁻¹) light intensities were simulated in a growth chamber. In these conditions, the expression of Scgst1 differed significantly between *S. commersonii* and *S. tuberosum* (SPV11) plants. In *S. commersonii*, Scgst1 mRNA accumulation progressed as the temperature was gradually lowered to 2°C either under low (LL) or high (HL) light intensity (Fig. 5a). In the same conditions, Scgst1 transcript did not accumulate in *S. tuberosum* (SPV11) plants. Similarly, in high light (HL) at freezing temperature (−3°C), Scgst1 transcript accumulated in *S. commersonii* but not in *S. tuberosum*. Lower amount of Scgst1 transcript was present in *S. commersonii* at freezing temperature under low light (LL) than high light (HL).

The expression of Scgst1 under low temperature (2 and −3°C) and high light (HL) (700 µmol m⁻² s⁻¹) conditions was studied also in the somatic hybrid SH9A and in three genotypes (1020, 2019, 2051) derived by selfing SH9A (Fig. 5b). The freezing tolerance of these four genotypes was in the range fROM −4.6 to −3.0°C (Table 2). The expression of Scgst1 was induced at non-freezing temperature (2°C) under high light (HL) conditions in the somatic hybrid but not in the other genotypes studied. However, at freezing temperature (−3°C) Scgst1 transcript did not accumulate in any of the genotypes studied.

Fig. 5. Effect of low temperature and light on the expression of Scgst1 gene in potato. Potato plants were either kept in control conditions or exposed to non-freezing (2°C) or freezing (−3°C) temperatures in low (LL) (80 µmol m⁻² s⁻¹) or high (HL) (700 µmol m⁻² s⁻¹) light conditions. The level of Scgst1 mRNA after exposure to 2 or −3°C in the presence of low (LL) or high (HL) light in a) *Solanum commersonii* (scmm) and *S. tuberosum* (SPV11), and b) in high light (HL) conditions in somatic hybrid SH9A, and in three genotypes of S1 progeny (1020, 2019, 2051). LT₅₀-values of non-acclimated plants are presented in Table 2. RNA blots were hybridized as described in Fig. 2 and in Section 2.
4. Discussion

Under field conditions, frosts that damage potato canopy, often develop rapidly so that the temperature decline is relatively fast. In addition, freezing stress is often combined with excessive light the following morning increasing the risk for oxidative stress. Subsequently, potato plants have a limited time to acclimate and sufficient freezing tolerance of non-acclimated plants or rapid response to decreasing temperature is required to avoid freezing damages. We have isolated and characterized a novel low temperature induced gene, Scgst1 (Solanum commersonii glutathione S-transferase) from a freezing tolerant wild potato species, S. commersonii. Strong induction of Scgst1 transcript was characteristic to S. commersonii but not to S. tuberosum in experimental conditions where the risk for a formation of active oxygen species (AOS) was increased. The Scgst1 sequence was present as a single copy in S. tuberosum genome, but did not exhibit the same cold induction properties as in S. commersonii. Similarly, in rice, the accumulation of cold regulated cDNA pBC591 (later identified as GST, GenBank™ Q06398) was also differentially regulated in freezing sensitive and tolerant rice genotypes [24]. Actually, Scgst1 expression pattern in the S1 genotypes resembled that of S. tuberosum. Hence, in addition to Scgst1 expression also other factors contributed to the observed freezing tolerance in the S1 lines. It may be that the interaction of S. commersonii and S. tuberosum genomes could contribute to the intermediate freezing tolerance and Scgst1 transcript abundance in the somatic hybrid SH9A. Similarly in wheat–rye interspecific combinations, the mRNA accumulation kinetics of cold inducible Wsc120 gene resembled more closely the freezing sensitive wheat parent [25]. The authors concluded that in the interspecific hybrids the gene dosage derived from the sensitive parent affects not only freezing tolerance but also the expression of cold induced genes.

Active oxygen species (AOS) and salicylic acid are known to induce GST levels [2]. It has also been reported that several environmental stresses such as drought, heat and low temperature can trigger the formation of AOS [26]. In the present study the transcription of Scgst1 was initiated rapidly in response to salicylic acid and hydrogen peroxide treatments. Significant accumulation of the transcript was also detected after heat stress but not after drought stress. Since theoretically, both heat and drought involve AOS formation, it can be speculated that the drought treatment might not have been severe enough to accumulate high amounts of AOS and induce Scgst1 expression to the extent as the heat treatment. Recently, it has been reported that the signaling pathway leading to adaptation to heat stress in potato involves salicylic acid and hydrogen peroxide [27]. In the case of heat stress, the induction of Scgst1 may thus be regulated both via salicylic acid and hydrogen peroxide. The observed fast response of Scgst1 transcription to hydrogen peroxide and salicylic acid indicates that these molecules might be involved in the early steps of the Scgst1 signaling pathway. The Scgst1 transcription was also intensively upregulated by continuos light at low temperature, i.e. in conditions where the formation of AOS is known to increase [28]. The response was specific to S. commersonii since the Scgst1 transcript did not accumulate in S. tuberosum plants subjected to the same conditions. It could be speculated that a faster and a more sensitive reception and transduction of the stress signal is characteristic to S. commersonii.

Due to a wide variety of substrates, GSTs are involved in numerous biochemical processes in the plant cell [2]. When the Scgst1 sequence was compared to other known GST sequences, the search revealed the highest homology to a type III GST, Hmgst, an auxin binding protein from Hyoscyamus muticus [5]. Scgst1 was not upregulated by treatments with auxins as Hmgst. At low temperature, substrates for Scgst1 could be, for example, lipid peroxidation products. A search for other low temperature regulated GSTs identified the maize Bz2 gene, which is defined as the last step of the anthocyanin biosynthesis pathway [7]. Thus the Scgst1 gene product may be involved in the detoxification of electrophilic compounds that accumulate as a result of active oxygen species. Rapid induction of Scgst1 transcription by hydrogen peroxide, salicylic acid and experimental conditions, which are known to cause formation of AOS, support this hypothesis.

Northern blot analysis revealed two Scgst1 transcripts (approx. 1.1 and 1.0 kb) in stress treated plants. However, only the amount of the smaller transcript was affected by stresses. Southern blot analysis revealed that Scgst1 is repre-
sented as a single copy in the genome. Thus, the bigger transcript was probably not a transcript of a highly homologous copy of another GST gene. In this study we are unable to further speculate the function of these two transcripts. However, in S. commersonii and SH9A total GST activity increased after 2 days of cold acclimation whereas in S. tuberosum the activity decreased. Hence, the accumulation of the smaller transcript of Scgst1 took place simultaneously with increased GST activity.

Adaptation to low temperature is based on numerous mechanisms of survival in freezing tolerant species. The polygenic nature of the trait becomes a challenge when the molecular basis is studied. Despite the complexity, there are several reports showing elevated levels of cold regulated gene transcripts in freezing tolerant genotypes [24,25]. In wheat and rye as well as in wheat–rye interspecific hybrids northern analysis showed mRNA accumulation of the low temperature induced gene Wsc120 in all of the genotypes [25]. A strong mRNA signal was sustained throughout the acclimation period in rye whereas the signal was reduced in wheat. In the present study, a strong mRNA signal and greater differences between freezing tolerant and sensitive species were detected when excess light was combined with low temperature. However, the association between freezing tolerance and the abundance of Scgst1 transcript was lost in the S1genotypes. Careful evaluation of the kinetics of gene expression in closely related genotypes expressing diverged freezing tolerance is required in order to obtain reliable knowledge concerning the importance of expression of specific genes on freezing tolerance.

Acknowledgements

This study was supported by the Academy of Finland, Finnish Cultural Foundation, Kemira and Agronomy Foundation, all of which are gratefully acknowledged. Tony H.H. Chen, Department of Horticulture, Oregon State University, is acknowledged for introduction to the subject of potato freezing tolerance and Terri Lo-max, Department of Botany and Plant Pathology, Oregon State University, is acknowledged for the help in the cloning work. Thanks are due to S. Grillo, CNR-IMOF, Portici, Italy and Shri Mo-

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