Functional screening of a cDNA library from the desiccation-tolerant plant *Selaginella lepidophylla* in yeast mutants identifies trehalose biosynthesis genes of plant and microbial origin

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**Abstract** Trehalose is a non-reducing disaccharide that accumulates to large quantities in microbial cells, but in plants it is generally present in very low, barely-detectible levels. A notable exception is the desiccation-tolerant plant *Selaginella lepidophylla*, which accumulates very high levels of trehalose in both the hydrated and dehydrated state. As trehalose is known to protect membranes, proteins, and whole cells against dehydration stress, we have been interested in the characterization of the trehalose biosynthesis enzymes of *S. lepidophylla*; they could assist in engineering crop plants towards better stress tolerance. We previously isolated and characterized trehalose-6-phosphate synthases from *Arabidopsis thaliana* (desiccation sensitive) and *S. lepidophylla* (desiccation tolerant) and found that they had similar enzymatic characteristics. In this paper, we describe the isolation and characterization of trehalose-6-phosphate phosphatase from *S. lepidophylla* and show that its catalytic activities are also similar to those of its homolog in *A. thaliana*. Screening of an *S. lepidophylla* cDNA library using yeast trehalose biosynthesis mutants resulted in the isolation of a large number of trehalose biosynthesis genes that were of microbial rather than plant origin. Thus, we suggest that the high trehalose levels observed in *S. lepidophylla* are not the product of the plant but that of endophytes, which are known to be present in this plant. Additionally, the high trehalose levels in *S. lepidophylla* are unlikely to account for its desiccation tolerance, because its drought-stress-sensitive relative, *S. moellendorfii*, also accumulated high levels of trehalose.

**Keywords** Desiccation tolerance · *Selaginella lepidophylla* · TPS · TPP · Trehalose

**Introduction**

Trehalose is a non-reducing disaccharide (α-1-glucopyranosyl-(1→1)-α-1-glucopyranoside) present in bacteria, yeast, fungi, invertibrates, and desiccation-tolerant plants. In these different organisms, trehalose functions as a reserve carbohydrate as well as an important stress-protecting molecule (Elbein et al. 2003). The hydrolysis of trehalose is a major event during fungal spore germination (Thevelein 1984) and insect flight (Becker et al. 1996), where trehalose presumably supplies glucose as a carbon and energy source. In mycobacteria and corynebacteria, trehalose is also an important structural component of the cell wall (Lederer 1976). Finally, trehalose, trehalose 6-phosphate (T6P; the intermediate molecule during trehalose biosynthesis), or the enzymes involved in its biosynthesis have also been shown to act as regulatory molecules, e.g., by controlling the influx of glucose into glycolysis in yeast cells (Bonini et al. 2003; Hohmann et al. 1996).

Desiccation-tolerant organisms are able to lose 80–95% of their water content and stay dormant in the dehydrated state until water becomes available (Crowe et al. 1992).
A common characteristic in most of these organisms is the rapid increase in compatible solutes, such as trehalose, during dehydration. High trehalose contents have been measured during desiccation in some ‘resurrection’ plants, yeast and bacterial cells, fungal spores, and also in microscopic animals such as nematodes, rotifers and, tardigrades (Hengherr et al. 2008; Oliver et al. 2000). In the course of desiccation, trehalose contributes to membrane stability by preventing the lipid phase transition and fusion of drying vesicles (Crowe 2007). Furthermore, the accumulation of trehalose in response to stress in Archaeabacteria suggests that the protective role of trehalose during cell dehydration might be an ancient adaptation that is evolutionarily preserved in desiccation-tolerant plants (Zaparty et al. 2013). Among vascular plants, high amounts of trehalose are detected in only a few desiccation-tolerant plants, such as *Myrothamnus flabellifolius*, *Sporobolus atrovirens*, and *Selaginella lepidophylla* (Müller et al. 1995).

*Selaginella lepidophylla* is a desiccation-tolerant plant from the Chihuahuan desert of North America. This lyco-phyte is able to lose 80–95 % of its protoplasmic water during a dry period and revive when water becomes available again (Yobi et al. 2012). During the dehydration–hydration cycles, the stems of *S. lepidophylla* curl and uncurl. Ultrastructural and biochemical analyses of the microphyll behavior during rehydration uncurling showed that the cellular and basal enzymatic integrity of the desiccated microphylls was conserved (Adams et al. 1990; Bergstrom et al. 1982; Yobi et al. 2013). Exceptionally for plants, the trehalose content in *S. lepidophylla* exceeds those of sucrose and glucose; however, in its desiccation-sensitive relative *Selaginella moellendorffii*, even higher levels of trehalose were detected (Yobi et al. 2012, 2013). We recently summarized the characteristics of *S. lepidophylla* that may be involved in its extreme drought-stress tolerance (Pampurova and Van Dijck 2014).

Because of the very low trehalose content in most vascular plants, they were thought to have lost the capacity to produce this molecule and that it was replaced by sucrose. However, most or all desiccation-intolerant vascular plants, such as *Arabidopsis thaliana* and *Oryza sativa*, harbor large trehalose-biosynthetic gene families in their genomes (Avonce et al. 2010; Leyman et al. 2001; Lunn 2007). In plants, trehalose biosynthesis involves a two-step pathway catalyzed by T6P synthase (TPS; EC 2.4.1.15) and T6P phosphatase (TPP; EC 3.1.3.12) (Avonce et al. 2006). In the first step, TPS catalyzes the transfer of glucose from UDP-glucose to glucose 6-phosphate forming T6P and UDP, while in the second step TPP dephosphorylates T6P to trehalose and inorganic phosphate (Elbein et al. 2003). Trehalose is catabolized by trehalase (EC 3.2.1.28) into two glucose moieties (Avonce et al. 2006). In the model plant *A. thaliana*, there are 11 genes encoding TPS- or TPS-like proteins, 10 encoding TPPs, and one encoding trehalase (Leyman et al. 2001).

Given the high trehalose content in *S. lepidophylla*, the characterization of its trehalose biosynthetic genes might provide insights into the evolutionary preservation of trehalose in a desiccation-tolerant plant. Until now, only one TPS-encoding gene (designated *SITPS1*) has been reported from *S. lepidophylla* (Márquez-Escalante et al. 2006; Valenzuela-Soto et al. 2004; Van Dijck et al. 2002). Similar to *ArTPS1*, this *SITPS1* is able to complement a yeast *tps1A* mutant growing on glucose, but only when its inhibitory N-terminal domain is removed (Van Dijck et al. 2002). The removal of this domain results in very high enzymatic activity upon expression in yeast (Van Dijck et al. 2002). One hypothesis that may support high trehalose levels in *S. lepidophylla* is that in this plant the N-terminal domain may be inhibited by a protein or metabolite or removed by a specific protease, resulting in high enzymatic activity. To investigate this possibility, we generated an *S. lepidophylla* cDNA library in a yeast expression vector. A large number of screenings in yeast resulted in the identification of many TPS-encoding genes that showed more homology with fungal TPS than with plant TPSs. Because *S. lepidophylla* harbors many endophytes (Brighigna et al. 2002) and is unable to regenerate from spores without them, i.e., when grown in sterile conditions (personal communication, G. Iturriaga), we here suggest that the high trehalose levels present in *S. lepidophylla* originate from the endophytes and are required for normal growth of *S. lepidophylla*.

Materials and methods

Plant material

Desiccated *S. lepidophylla* plants were obtained from Prof. G. Iturriaga (Centro de Investigación en Biotecnología—UAEM, Morelos, Mexico) and from the companies Floréac (Lochristi, Belgium) and Livo B.V. (Overijssel, The Netherlands). To construct the cDNA library, plant samples were taken at four different time points during a rehydration/dehydration cycle. *Selaginella lepidophylla* plants used for real-time quantitative reverse transcription PCR (RT-qPCR) analysis were completely hydrated (4 days hydration after the initial rehydration/dehydration cycle) and completely dehydrated (3 days dehydrated after the initial rehydration/dehydration cycle).

*Selaginella lepidophylla* cDNA library construction

RNA samples were collected from fully-desiccated, hydrated (24 h after watering), partly-dried plants (8 h...
drying), and completely-dried plants (24 h drying). RNA extraction was performed as previously described (Valenzuela-Avendano et al. 2005). Complementary DNA was generated using the cDNA library construction kit (Invitrogen, Carlsbad, CA, USA), cloned in the Gateway donor vector pDONR222, and transferred to the yeast expression vector pVV214 (Van Mullem et al. 2003). The resulting cDNA library was composed of 1.019 × 10^{11} colony forming units ml^{-1} with an average cDNA insert size of 1.16 kb.

Isolation of proteins interacting with the N-terminus of STPS1

Expression of STPS1 in a yeast tps1Δ mutant does not restore growth on glucose (Van Dijck et al. 2002). However, expression of a construct in which the first 300 nucleotides (100 amino acids) of STPS1 were removed resulted in the production of a protein with high enzymatic activity and was able to restore the mutant’s growth on glucose. To identify proteins that might interact with the N-terminus and remove its inhibitory activity on the catalytic domain, we transformed the S. lepidophylla cDNA library into a Saccharomyces cerevisiae strain (Gietz et al. 1995) expressing the full-length STPS1 and selected transformants that could grow on glucose. Plasmids from positive clones were isolated, amplified in E. coli, and retransformed into the same yeast strain for confirmation. The inserts of the positive clones were sequenced.

Isolation of TPP and TPS genes from S. lepidophylla cDNA library

To isolate TPP and TPS genes, we transformed the cDNA library into yeast tps1Δ or tps2Δ strains, which lack TPS or TPP activity, respectively. Complemented transformants that could grow on glucose (tps1Δ) or at 38 °C (tps2Δ) were retested in a spot assay. For the spot assays, overnight liquid cultures were adjusted to an optical density at 600 nm (OD_{600}) = 0.1, diluted tenfold, and spotted (5 μL) on synthetic solid medium without uracil and with glucose or galactose as carbon source. Transformants in the tps1Δ background were selected for growth on glucose, and transformants in the tps2Δ background were selected for growth at 38 °C. Plasmids from positive clones were isolated, amplified in E. coli, and retransformed into the same yeast strain for confirmation. The inserts of the positive clones were sequenced.

In an alternative approach to isolate these genes, we used the degenerate-oligonucleotide PCR approach. Based on sequences obtained from searches of the S. lepidophylla EST library (Iturriaga et al. 2006) and homologous TPS and TPP genes from other species, degenerate primers (see Table S1) were designed and combined with primers specific to the AttB-sites on the pVV214 vector. After obtaining specific cDNA fragments, a second set of primers was designed to obtain the complete cDNA sequence. The following PCR program was used: initial denaturation at 95 °C for 5 min, five cycles of (95 °C for 1 min, 30 °C for 1 min, 72 °C for 2 min), 30 cycles of (95 °C for 1 min, 55 °C for 1 min, 72 °C for 2 min), and final extension step at 72 °C for 7 min. To obtain the full cDNA sequence with the specific primers, after initial denaturation for 5 min at 95 °C, the reaction was as follows: 35 cycles (95 °C for 40 s, 65 °C for 1 min, 72 °C for 1 min) and final extension at 72 °C for 7 min.

Phylogenetic analysis

The sequences used for the phylogenetic reconstruction of the TPP gene family were collected using the PLAZA 1.0 platform (Proost et al. 2009) as previously described (Avonce et al. 2010). The gene families from Physcomitrella patens, A. thaliana, and S. moellendorffii were included in the analysis (supplementary Table 2). Multiple sequence alignment of the deduced protein sequences was performed with Clustal Omega (Sievers et al. 2011), and the distance matrix with the neighbor-joining algorithm. The estimated phylogenetic trees were drawn with NJplot (Perri`ere and Gouy 1996).

Growth conditions, yeast strains and transformation

Yeast cells were grown in synthetic growth medium (1.7 g Bacto-yeast nitrogen base without amino acids (pH 6.0), 5 g ammonium sulfate; supplemented with 50 mg adenine, 100 mg histidine, 250 mg leucine, 100 mg tryptophan, and 50 mg uracil in 1 L) and 2% galactose (SGal) or 2% glucose (SGlc). The yeast strains were: wild-type (WT), W303-1A (Mata ade2-1 trp1-1 his3-11, 15 ade2-1 can1-100 GAL SUC2) (Thomas and Rothstein 1989); tps1Δ, YSH290 (W303-1A, tps1Δ::TRP1) (Hohmann et al. 1993); and tps2Δ, YSH488 and YSH587 (W303-1A, tps2Δ::LEU2) (Neves et al. 1995). Shuttle vectors pSAL4 (Zentella et al. 1999) and pYX212 (Novagen) were used to express the genes of interest in yeast. Yeast transformation was performed according to Eibl (1992), and transformants were selected on plates containing minimal medium without uracil.

Trehalose measurements

Trehalose was measured as previously described using Humicola sp. trehalase and glucose oxidase/peroxidase assays (Zentella et al. 1999).
TPP activity

TPP activity was determined as previously described (De Virgilio et al. 1993; Zentella et al. 1999). Protein concentrations were measured with the Bradford method (Bradford 1976). Enzyme activity is expressed as nanokatals per gram of protein (nkat g protein⁻¹).

qRT-PCR analysis

Gene expression was tested with RT-qPCR. RNA was extracted according to Valenzuela-Avendano et al. (2005), and cDNA was prepared from 2 µg of total RNA using a commercial cDNA synthesis kit (Invitrogen) before analysis on an ABI PRISM 7000 thermocycler using Platinum SYBR Green qPCR SuperMix UDG (Invitrogen) according to manufacturer’s instructions. All reactions were done in triplicate. Relative expression levels of target genes were calculated using Student’s t test.

Results

Initial screening of S. lepidophylla cDNA library for TPS and TPP genes

We used S. cerevisiae tps1A and tps2A strains to isolate TPS or TPP genes from the desert resurrection plant S. lepidophylla. A total of 30,000 transformants was screened for growth on glucose (tps1A complementation) and at 38 °C (tps2A complementation). Plasmid DNA from positive transformants was isolated and retested. Interestingly, screening for genes that suppressed the glucose growth defect of the tps1A strain led to the identification of several genes with higher sequence similarities to fungal TPS than to plant TPS proteins (Table 1). Furthermore, the positive clones that allowed growth at 38 °C of the tps2A strain harbored genes encoding stress proteins, such as LEA proteins and dehydrins, instead of the expected TPS genes.

Isolation of active microbial TPS genes from the S. lepidophylla cDNA library

According to Adams et al. (1990) the content of trehalose in S. lepidophylla is much higher than in other plants. We have previously shown that an N-terminal truncation of SITPSI (or AtTPSI) results in a much higher enzymatic activity and very good complementation of the yeast tps1A mutant (Van Dijck et al. 2002). One possibility for the high trehalose

| Growth conditions | Strain | Gene | Species | Kingdom |
|------------------|--------|------|---------|---------|
| Glucose          | tps1A  | TPS  | Coniosporium apollinis | Fungi |
| Glucose          | tps1A  | TPS  | Polysphondylium pallidum | Amoebozoa |
| Glucose          | tps1A  | TPS  | Leptosphaeria maculans | Fungi |
| Glucose          | tps1A  | TPS  | Exophiala dermatitidis | Fungi |
| 38 °C            | tps2A  | DHN  | Selaginella lepidophylla | Plantae |
| 38 °C            | tps2A  | LEA  | Selaginella lepidophylla | Plantae |
| 38 °C            | tps2A  | ELIP | Selaginella lepidophylla | Plantae |
| 38 °C            | tps2A  | GST  | Selaginella lepidophylla | Plantae |

TPS T6P synthase, DHN dehydrin, LEA late embryogenesis abundant protein, ELIP early light inducible protein, GST glutathione-S-transferase

a The closest homologs to the isolated TPS genes are from the indicated species
b The isolated genes are complete matches with already-identified genes from S. lepidophylla
Isolation and characterization of *S. lepidophylla* TPPs

*SITPP* genes were not isolated during the *S. lepidophylla* library screenings, so a degenerate-oligonucleotide-based PCR approach was used. Degenerate oligonucleotides were designed according to sequences from the available *S. lepidophylla* EST library and from *TPP* genes from other plant species. Based on the sequences of the primary PCR products, a second set of primers was designed and used in combination with primers annealing to the vector sequence to amplify the complete cDNA inserts. Three *SITPP* genes, named *SITPPA*, *SITPPB*, and *SITPPC*, were isolated. To phylogenetically characterize them, the *TPP* gene families from three selected species were obtained from PLAZA 1.0 (Proost et al. 2009): *Physcomitrella patens* had eight *TPP* genes (PP00032G01010, PP00046G00150, PP00047G00570, PP00056G01260, PP00141G00660, PP00201G00540, PP00227G00210, and PP00333G00270), *S. moellendorffii* had two (SM00030G00440 and SM00032G01340), and *A. thaliana* had 10 (AT1G22210, AT1G35910, AT1G78090, AT2G22190, AT4G12430, AT4G22590, AT4G39770, AT5G10100, AT5G51460, and AT5G65140). According to our phylogenetic analysis, *SITPPA* and *SITPPC* were orthologous *SM00032G01340* and *SM00030G00440*, respectively, inferring a common
ancestor gene prior to the speciation event separating *S. moellendorffii* and *S. lepidophylla*. The *SITPPB* gene did not have an ortholog in *S. moellendorffii* (Fig. 2a).

To investigate the functional activity of the *SITPPA* (pI 5.36, 45.87 kDa) and *SITPPB* (pI 5.65, 34.39 kDa) proteins, the genes were cloned into pSal4 and pYX212 vectors.
Phylogenetic analysis with homologous halose content for SlTPPA and SlTPPB, with 52 trehalose measurements indicated a subtle increase in trehalose levels and TPP enzymatic activity of wild type strains, respectively. This correlated with a measurable enzymatic activity could be measured. Compared with the control tps2 strain, in which no TPP enzymatic activity could be measured. Compared with the 799.6 nkat g protein−1 measured in the WT strain, the enzymatic activities obtained with the S. lepidophylla TPP genes were low (Table 2).

Isolation of an S. lepidophylla class II TPS gene

Apart from the active TPS and TPP enzymes (SITPS1 and SITPPA, SITPPB, and SITPPC in S. lepidophylla), all plants also have genes that encode for so-called class II TPS enzymes. They are very similar to the class I active TPS enzymes but lack the N-terminal extension, and some crucial amino acids in the catalytic domains are not conserved. Most species have more class II than class I enzymes (e.g., A. thaliana has four and seven, respectively); only in the primitive alga Ostreococcus tauri is there an equal number (one each) (Avonce et al. 2010). The S. lepidophylla EST library (Iturriaga et al. 2006) screening resulted in the isolation of a putative SITPS class II fragment. Based on this fragment, specific primers were designed (supplementary Table 1), and the SITPS class II cDNA clone was amplified. This class II enzyme had 849 amino acids and, similar to the class II proteins of other plants, could not complement yeast tps1 or tps2 mutants (data not shown). Phylogenetic analysis with homologous TPS class II genes from A. thaliana, P. patens, and S. moellendorffii (Fig. 3a) indicated that the closest homolog for SITPS class II was the TPS class II SM0003G06280 gene from S. moellendorffii (88.9 % identity). The second TPS class II gene from S. moellendorffii, SM0057G00180, had 71.84 % identity with our SITPS class II gene. Shared identity with the four P. patens TPS II genes was 73.37, 74.46, 69.14 and 70.93 % for PP0081G00980, PP00088900870, PP00080G00530, and PP00153G00600, respectively. Among the seven AtTPS class II genes, SITPS class II shared closest homology with AtTPS5 (67.78 % identity, AT4G17770). Because the whole genome sequence is not yet available, how many class II enzymes there are in S. lepidophylla is not clear.

Relative transcript levels of SITPS1, SITPPA, and SITPPB during hydrated and dehydrated states of S. lepidophylla

The expression levels of three trehalose biosynthetic genes were measured. The expression level of SITPS1 did not change significantly between the hydrated and dehydrated states of S. lepidophylla (Fig. 4). In the case of the TPP genes, SITPPA expression was significantly decreased during dehydration, while there was no significant difference in the expression level of SITPPB between the two states. As mentioned above, trehalose levels do not differ significantly between the hydrated and dehydrated states of this species (Adams et al. 1990; Yobi et al. 2013).

Discussion

The high trehalose levels in S. lepidophylla have always been linked to its high drought-stress tolerance. One of the first genes isolated from this plant was SITPS1, encoding a trehalose-6-phosphate synthase (Zentella et al. 1999). However, heterologous expression in yeast showed that this enzyme was even less active than AtTPS1 (Van Dijck et al. 2002). If trehalose biosynthesis was linked to drought-stress tolerance, one would expect the enzymatic activity and trehalose levels to be higher in the dry state. However, SITPS1 is active in both the dry and hydrated states, with fully-hydrated plants having the highest SITPS1 activity (Márquez-Escalante et al. 2006). Additionally, the levels of trehalose were not significantly different between the dry and hydrated states. The level of trehalose in S. lepidophylla corresponded to the levels measured in microbes, which are 3,000 times higher than in plants such as rice, tomato, and potato. Although high trehalose levels have been linked specifically with the desiccation-tolerance of S. lepidophylla, a large-scale comparative metabolomics study revealed that S. moellendorffii, the
drought-sensitive relative of *S. lepidophylla*, had even higher levels of trehalose than *S. lepidophylla* (Yobi et al. 2012). The high trehalose levels detected in both lycophytes represent an evolutionary-preserved metabolic feature that requires further study. The results obtained here question the origin of trehalose in *S. lepidophylla*, because the *S. lepidophylla* cDNA library screenings resulted in the isolation of T6P synthases of microbial origin (Table 1; Fig. 1a, b). This finding may explain the similar trehalose concentrations in the hydrated and dry states. Furthermore, ultrastructural analysis of *S. lepidophylla* during rehydration clearly showed the presence of fungi and bacteria in the dry microphylls (Brighigna et al. 2002). These facts, together with the isolation of TPS genes of microbial origin and the
were orthologs of two SlTPPC genes
The phylogenetic analysis showed that SlTPPA (Fig. 2a). The complementation of the yeast S. moellendorffii SlTPPA genes proved that both tps2 genes strongly support the hypothesis that trehalose in this plant is of microbial origin. Recently, the S. moellendorffii genome was shown to also contain DNA from nucleocytoplasmic large DNA viruses, probably a result of horizontal gene transfer (Maumus et al. 2014). The endogenous origin of trehalose in S. lepidophylla could be proven by measuring trehalose content in sterile in vitro cultures of the plant. However, attempts to grow S. lepidophylla in vitro from spores have failed, probably because of the lack of symbiotic bacteria/fungi necessary for this lycophyte to grow (G. Iturriaga, personal communication), further supporting the microbial origin of trehalose production.

As the cDNA library screening was not successful in isolating SITPP genes, degenerate-oligonucleotide PCR approaches were used to isolate three SITPP genes (SITPPA, SITPPB and SITPPC) from the cDNA library. The phylogenetic analysis showed that SITPPA and SITPPC were orthologs of two S. moellendorffii genes, suggesting common ancestor genes prior to speciation, while SITPPB did not appear to have an ortholog in S. moellendorffii (Fig. 2a). The complementation of the yeast tps2Δ mutant and enzymatic activity determinations proved that both SITPPA and SITPPB were active TPP proteins, although their activity (nkat g protein⁻¹) values were very low compared with that of ScTPS2 (WT control) and slightly higher than that of the tps2Δ strain (the negative control) (Table 2) (SITPPC was not tested in this assay). The low enzymatic activity of the SITPP enzymes did not differ significantly from that of AtTPPB, which was used as a control (Fig. 2b; Table 2). Again, the data indicate that the basic enzymatic activity of TPP in S. lepidophylla did not differ from that of other plants, so it probably cannot account for the higher presence of trehalose.

Finally, similar to all other plant species, S. lepidophylla has at least one class II enzyme, which we isolated using a PCR approach. This means that all three groups of enzymes (or regulatory proteins in the case of the class II protein) are present in this resurrection plant. Sequencing of the S. lepidophylla genome would provide an opportunity to characterize the complete trehalose biosynthetic gene family in this desiccation-tolerant plant. In the case of S. moellendorffii, in silico evolutionary studies of the gene family are possible, because its genome has already been sequenced (Banks et al. 2011), and in silico studies of other gene families have already been performed (Saha et al. 2013). Evolutionary studies of the S. lepidophylla trehalose biosynthetic genes might provide important hints into their specific functions under different conditions. Our expression analysis already suggests diverse regulation of SITPPA and SITPPB during hydration and dehydration. SITPPA transcript levels were significantly lower during dehydration, while the expression of SITPPB did not change significantly during this process.

The fact that S. moellendorffii has similarly high trehalose levels to S. lepidophylla but is drought-stress sensitive seems to suggest that other characteristics (maybe together with trehalose) are involved in the latter’s desiccation tolerance. An overview of these characteristics can be found in the review by Pampurova and Van Dijck (2014).

Conclusions

The isolation and characterization of three SITPPs genes and one SITPS class II gene, together with the previously characterized SITPS1, showed that this plant also has all three groups of trehalose biosynthesis enzymes. The most striking result of our work was the isolation of many TPS genes of microbial origin from our cDNA library. Additionally, the S. lepidophylla TPPA and TPPB enzymes were not more active than A. thaliana TPP. Taken together, the results suggest that the high trehalose content in this plant (and probably also in S. moellendorffii) is of microbial origin.

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