Cloning and characterization of a salt stress-inducible small GTPase gene from the model grass species *Lolium temulentum*

James E. Dombrowski, James C. Baldwin, Ruth C. Martin

USDA-ARS, National Forage Seed Production Research Center, Oregon State University, 3450 SW Campus Way, Corvallis, OR 97331, USA

USDA-ARS Cereal Crops Research Unit, 501 Walnut Street, Madison, WI 53726, USA

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Summary

A gene encoding a small guanosine triphosphate (GTP)-binding protein (smGTP) related to the Rab2 gene family of GTPases was identified during the analysis of a salt stress suppression subtractive hybridization (SSH) expression library from the model grass species *Lolium temulentum* L. (Darnel ryegrass). The smGTP gene was found to have a low-level constitutive expression and was strongly induced by salt stress in root, crown and leaf tissues. The expression pattern of the smGTP gene was compared against two additional stress genes identified in the SSH expression library, the well-characterized dehydration stress tolerance gene, delta 1-pyrroline-5-carboxylate synthetase (P5CS), and the cold response gene COR413. The genes were analyzed for their response to salinity as well as their responses to 7 different forms of abiotic stress in *L. temulentum* plants. The smGTP gene displayed an expression pattern similar to the P5CS gene, suggesting a role in dehydration stress. In contrast, the COR413 gene was found to be up-regulated in response to all stresses tested and has utility as a general stress marker in grass plants.

Introduction

Over the past few decades there has been a dramatic increase in the salinization of arable land (Wang et al., 2003; Munns, 2005). As land becomes more limited for conventional agriculture, plants grown on marginal soils will be exposed to higher levels of soil salinity. Forage grasses are a critical
component of feed used in livestock production worldwide, with many of these same species of grasses being utilized for lawns, erosion prevention and recreation. Soil salinity is one of the major abiotic stresses responsible for reduced persistence, yield and biomass accumulation in many crops, including forage. Consequently, it is of great importance to develop a better understanding of salt tolerance in forage species. Forage grass species are generally polymorphic, obligate out-crossers that are self-incompatible (Huff, 1997; Kölliker et al., 1999; Roldán-Ruiz et al., 2000; Kubík et al., 2001). Because of their high genetic diversity the mechanisms of salt tolerance are poorly understood. Fortunately, Lolium temulentum L. (Darnel ryegrass) lacks many of the negative genetic attributes associated with most other grass species. L. temulentum is better suited for genetic and molecular genetic analyses than related grasses. L. temulentum is a diploid self-fertile species with a short life cycle (2–3 months) (Evans, 1958, 1999). Furthermore L. temulentum has also been shown to be able to be out-crossed with other Lolium species, making it an ideal model system for forage and turf grass research (Thorogood and Hayward, 1992; Thomas et al., 1999; Yamada, 2001; Wang et al., 2005).

Over the past few years, considerable progress has been made towards understanding the mechanisms leading to salt tolerance in dicot species (Hasegawa et al., 2000; Seki et al., 2001; Ma et al., 2006). Unfortunately much less progress has been made in the study of salinity tolerance in monocot species. Although homologs of genes known to be involved in salt tolerance have been identified in rice (Kawasaki et al., 2001; Chao et al., 2005; Wu et al., 2005) and barley (Atlienza et al., 2004; Ueda et al., 2004) and recently in L. temulentum (Baldwin and Dombrowski, 2006), very little is currently known on the molecular mechanisms used by forage grasses for salt tolerance.

Since plants are sessile, they have developed mechanisms that enable them to sense stresses and to elicit complex interactions between signaling molecules and pathways to adapt to various stresses. In response to salt or dehydration stress, small molecules such as abscisic acid (ABA) and calcium are utilized by the plant to induce various signaling cascades. These pathways use various proteins such as phospholipases, kinases, calmodulin, calcium-binding proteins and transcription factors to activate genes necessary for water-related stress tolerance (for reviews, see Xiong et al., 2002; Chinnusamy et al., 2004; Munns, 2005; Yamaguchi-Shinozaki and Shinozaki, 2006). Another family of signaling proteins involved in many aspects of cellular maintenance and plant development are the GTPases which are grouped into two major classes, the small GTPases and the heterotrimeric G proteins (Assmann, 2002; Yang, 2002). The small GTPase superfamily is divided into at least 5 families including Ras, Rho, Arf, Ran and Rab (Takai et al., 2001; Yang, 2002). The majority of known small GTPases are involved in vesicle transport (Molendijk et al., 2004). Less is known concerning the role of small GTPases in stress-related signaling. Plant Ras-related G proteins have been shown to be mobilized by hormones, pathogen elicitors and abiotic stresses (Gu et al., 2004; Nibau et al., 2006). A small GTPase in the Rab5 family identified in the ice plant and jojoba have been shown to be up-regulated in response to salt stress (Bolte et al., 2000; Mizrahi-Aviv et al., 2002). The OsRab7 gene from rice was induced during treatments with ABA and salt stress (Nahm et al., 2003). When the AtRab7 gene was over-expressed in Arabidopsis the transgenic plants showed increased tolerance to salt and osmotic stress and reduced accumulation of reactive oxygen species during salt stress (Mazel et al., 2004).

In plants, Rab2 genes have been implicated in a variety of cellular, developmental and stress responses; however, the precise role and function of these Rab2 proteins remains to be determined. The tobacco NtRab2 and Arabidopsis AtRab2 have been shown to be involved in protein transport associated with the secretory pathway and are highly expressed in pollen and germinating seedlings (Moore et al., 1997; Cheung et al., 2002). In Lotus japonicus, the LjRab2 gene may be involved in the development of root nodules (Borg et al., 1997). In monocots, a Rab2 gene was implicated to have a role in desiccation tolerance and damage repair of membranes caused during desiccation and rehydration in the desiccation-tolerant grass Sporobolus stapfianus (SsRab2) (O’Mahony and Oliver, 1999). In rice, a Rab2 ortholog was found to be actively expressed at stages of cell division and elongation (Kim et al., 1997). Recently a Rab2A isoform was isolated from sugarcane and was postulated to be involved in the regulation of the transport of proteins essential for cells to divide and develop (Zhang et al., 2006).

Our laboratory previously identified a gene encoding for a small guanosine triphosphate (GTP)-binding protein (smGTP), related to the Rab2 gene family of GTPases, during the analysis of a salt stress suppression subtractive hybridization (SSH) expression library in L. temulentum (Baldwin and Dombrowski, 2006). The expression pattern of the smGTP gene was compared to two known stress genes also identified in SSH expression
library, the delta 1-pyrroline-5-carboxylate synthetase (P5CS) and the COR413 gene. These genes were analyzed for their response to different concentrations of salinity as well as their responses to 7 different forms of abiotic stress in *L. temulentum* plants. In this report we present evidence that *smGTP* plays a role in dehydration stress.

**Materials and methods**

**Plant materials**

*L. temulentum* L. (Darnel ryegrass) cv. Ceres seeds were planted in 4 in square pots (volume approximately 750 mL) in SB40 Sunshine Growing Mix (Sun Gro Horticulture, Canada) or in two layers of vermiculite: fine (top) and coarse (bottom). Plants were fertilized weekly using Technigro 20-18-20 all-purpose fertilizer (Sun Gro Horticulture, Canada). Plants were grown in a Conviron E15 (Conviron, Winnipeg, Canada) growth chamber under an 8 h photo-period at 21 °C day and 18 °C night. Initiation of flowering was performed by adjusting the growth chamber photo-period to 16 h days (long days) and 8 h nights. Mature flowering plants were grown for an additional 3–4 weeks under long days. As the seed heads entered dough stages, watering was reduced to every other day. Seed heads were allowed to ripen until most seed heads were at or beyond the hard dough stage of maturity prior to harvesting.

**Cloning of Lt-smGTP-binding protein and Lt-COR413 genes**

Rapid amplification of cDNA ends (RACE) was used to isolate the 5′ and 3′ regions of the small GTP binding protein (*S7SLT-C63*) and the *COR413* (*S7SLT-C459*) partial clones identified by polymerase chain reaction (PCR)-based subtractive suppression hybridization analysis (Baldwin and Dombrowski, 2006). Total RNA from leaf/crown tissues of *L. temulentum* (Baldwin and Dombrowski, 2006) represents the delta 1-pyrroline-5-carboxylate synthetase (*P5CS*) and the *COR413* protein (*S7SLT-C63*) and the kit 5′ primer. The 552 bp PCR product was ligated into the p-GEM-T Easy vector (Promega), cloned and sequenced.

For 3′ RACE, the Invitrogen 3′ RACE system was used according to the manufacturer’s recommendations. First-strand cDNA synthesis was performed using the kit’s adapter primer. Amplification of the *smGTP* cDNA was done using the AUAP primer from the kit and a gene-specific primer (5′-CTGGTGAGCTCAGCTTCCCTCCCAAGGC). The 387 bp PCR product was cloned into the p-GEM-T Easy vector (Promega) and sequenced. Amplification of the *COR413* cDNA was done using the AUAP primer from the kit and a gene-specific primer (5′-TCGGCCC-GTGCATGTCTCCAGTTCTGTG-3′) followed by nested PCR with a gene-specific primer (5′-TTCCGGGTGA-TTTGTATTTGCTGCCTCCGG-3′) and the kit AUAP primer. The 215 bp PCR product was cloned into the p-GEM-T Easy vector (Promega) and sequenced.

**Analysis of differentially expressed genes**

The sequences were subjected to several BLAST, BLASTX and RPS-BLAST searches against Genbank and the CDD databases to provide annotation information and ortholog sequences from other species (Altschul et al., 1997; Marchler-Bauer et al., 2003). This information was used to construct an alignment (Altschul et al., 1990). Closely related sequences were used for phylogenetic analysis and neighbor joining tree construction using the Mega 3.1 program (Kumar et al., 2004).

**Salt treatments**

Plants were grown in vermiculite for 8 weeks and treated with 500 mL of 500 mM sodium chloride (NaCl). Leaf, crown and root tissues were collected at 0, 12 and 24 h post treatment, immediately frozen in liquid nitrogen and stored at −80 °C.

**Time course analysis**

Plants were grown in soil for 6 weeks and then treated with 500 mL of 500 mM NaCl. The aerial portions of 6–7 plants were collected at 0, 1, 2, 4, 8, 12, 16 and 24 h post treatment, immediately frozen in liquid nitrogen and stored at −80 °C.

**Salt concentration analysis**

Plants were grown in soil for 6 weeks. Each pot containing 10–14 plants was treated with 500 mL of one of the following: 0, 100, 200, 300, 400 or 500 mM NaCl salt solution. The aerial portions of 6–7 plants were collected at 0, 12 and 24 h, immediately frozen in liquid nitrogen and stored at −80 °C.

**Stress treatments**

Plants were grown for 6 weeks as described above. Pots containing 12–16 plants/pot were then used for various stress treatments described below.
Drought: To induce drought stress, watering was stopped and pots were allowed to dry out overnight. After 24 h some very mild wilting was observed. Tissue was collected 12 h later.

Ultraviolet (UV) stress: Plants were laid on their side and irradiated for 5 min. Two hand-held 254 nm UV short-wave devices (Model UVG-11, Ultra-violet Products Inc., USA) were held 5–6 in above the stems and leaves. Tissue limpness was observed after irradiation and samples of 6–8 plants were collected 1 and 8 h post treatment.

Heat stress: Plants were subjected to 40°C in a Conviron E15 Growth Chamber to simulate heat stress. The plants were well watered and placed in a shallow pan of water to maintain adequate hydration during heat stress treatments. Tissue of 6–8 plants was collected after 2 and 8 h of heat stress.

Wounding: Plants were mechanically wounded by closing a hemostat perpendicularly across the leaves and stems 3–5 times. Tissue was collected 12 h after wounding.

Salt stress: Plants were subjected to salt stress by treating the soil with 500 mL of 500 mM NaCl. Plants showed signs of mild wilting in the leaf blades after 1 h. The aerial portions of 6–7 plants were collected 12 and 24 h after salt treatment.

Osmotic stress: Plants were subjected to osmotic stress by treating the soil with 500 mL of 12.87% polyethylene glycol (PEG) 6000 solution. Plants showed mild wilting of leaf blades after 1 h of treatment. The aerial portions of 6–7 plants were collected after 12 and 24 h of stress treatment.

Cold stress: Plants were subjected to 4°C for 24 h. Tissue was collected from plants after 24 h in the cold.

Control tissue was collected from plants that were untreated and watered normally. All tissues were collected, immediately frozen in liquid nitrogen and stored at –80°C.

Seed head development and post-harvest treatments

Seed heads were collected at different stages of development: emerging head – inflorescence emerging from flag leaf sheath, late to post anthesis – flowers emerged and senescing, milk to dough stage – green seed heads, and ripe seed head – stalk leaves green to yellow brown.

Harvest treatments were performed to simulate conditions that are commonly observed in grass seed production. Watering was withheld from the mature seed bearing plants for 24 h prior to the harvest date. Grass stalks were cut near ground level and stacked 15–20 cm high in a plastic tray to minimize water evaporation from the edges of the pile, while permitting evaporation from the top of the pile. Tissue samples were collected at 0, 12, 24 and 48 h after cutting. At these time intervals grass samples were taken from the cross-section (from top to bottom) of the stack, the seed heads were removed, and the remaining stems and leaf tissue collected. Each tissue sample was quickly cut into 1–2 in segments, packed in foil, frozen in liquid nitrogen and stored at –80°C until use.

RNA gel blot analysis of genes

Genes coding for P5SC (accession # EB709850), COR413 (accession # 891238) and small GTP binding protein (accession # 890880) were subjected to further analysis by RNA blot analysis. Harvested tissue was ground to a powder in liquid nitrogen using a precooled mortar and pestle. Total RNA extractions from these ground tissues were performed using TRIzol reagent (Life Technologies, Gaithersburg, MD, USA) following the manufacturer’s instructions. Ten micrograms of total RNA isolated from selected/treated L. temulentum plant tissues was electrophoretically separated on 1.2% denaturing formaldehyde agarose gels and blotted onto a Hybond N nylon membrane (Sambrook et al., 1989). Inserts of selected cDNAs were amplified using T7 and SP6 primers and a standard 3 step PCR protocol with an annealing temperature of 55°C. The amplified fragments were ethanol precipitated and rehydrated at a concentration of 25 ng per μL. Amplified fragments were used to generate 32P-labeled DNA probes using the Ambion DECaprime II DNA labeling kit (Ambion, USA) for RNA blot analysis. The membranes were hybridized overnight with a 32P-labeled probe in a solution containing 50% formamide, 5 × Denhart’s solution, 0.1% (w/v) sodium dodecyl sulfate (SDS), 6 × SSPE and 100 μg/mL denatured salmon-sperm DNA at 42°C. Filters were initially washed in 2 × SSC and 0.1% SDS at 42–50°C for 30 min, then subjected to a wash starting at 50°C and allowing to cool to RT with shaking, and briefly in 0.2 × SSC and 0.1% SDS at RT before autoradiography.

Results

Cloning and sequence analysis of the smGTP gene from L. temulentum

SSH was used to identify differential expression of genes related to salt stress tolerance in the model grass species L. temulentum (Baldwin and Dombrowski, 2006). Analysis of SSH library revealed a partial clone for a small GTP binding protein. RACE PCR was used to isolate the 5’ and 3’ ends of this clone. Sequence analysis showed a full-length cDNA of 1113 nucleotides, containing an open reading frame (ORF) of 633 nucleotides (start codon, 167–169; stop codon, 797–799). The ORF encoded a protein of 210 amino acids, with a potential molecular mass of 23 kDa (Figure 1). Protein similarity searches were performed with the BLAST program (Altschul et al., 1990). The protein sequence was most closely related to a small GTP-binding protein from Triticum aestivum (92% identity). The protein contained highly conserved motifs typical of the GTPase superfamily (Figure 1, boxed residues). Domains G1–G3 are involved in binding magnesium and GTP phosphate...
residues and G4 and G5 are involved in binding the guanine moiety (Pai et al., 1989; Bourne et al., 1991). Within this superfamily, the sequence most resembles the Rab family of small GTPases and contains sequence motifs typical of this family of proteins (Figure 1). Domains RabF1, RabF3 and RabF4 have been shown to be essential for the formation of the Rab:Rab Escort Protein (REP) complex which is then recognized by the Rab geranylgeranyl transferase (Pereira-Leal and Seabra, 2000, 2001). The translated protein sequence also contained a double cysteine prenylation motif at the C terminal of the protein to which the geranylgeranyl groups are transferred (also highlighted in gray and italicized).

Response of smGTP to salt stress

Since the smGTP gene was initially isolated from the aerial portion of salt-stressed L. temulentum plants, we wanted to investigate the tissue-specific expression of the gene. Plants were subjected to 12 h of salt stress and total RNA was extracted from root, crown and leaf tissue. RNA blot analysis was used to determine the salt-induced tissue expression pattern of the smGTP gene. As shown in Figure 3, the smGTP gene displayed low-level constitutive expression in all tissues prior to salt stress. After 12 h exposure to salt stress the smGTP...
gene showed increased transcript accumulation in all tissues tested.

To determine if the isolated smGTP gene has a role in salt stress, we decided to compare its expression pattern against two other genes identified during the analysis of a salt stress SSH expression library in L. temulentum (Baldwin and Dombrowski, 2006). The first gene is an ortholog of the wheat COR413 gene (84% similarity) encoding for a membrane protein that may act as a putative G-protein-coupled receptor. The COR413 gene is believed to be involved in either stress signaling or membrane stability during cold stress (Breton et al., 2003; Garwe et al., 2003). Another stress gene identified was the P5CS gene which encodes a key enzyme in proline biosynthesis (Hu et al., 1992). Proline is an important osmoprotectant produced in plants in response to water-related stresses (Delauney and Verma, 1993; Yoshiba et al., 1997; Kishor et al., 2005). P5CS has previously been shown to be involved in the biosynthesis of compatible solutes in response to salt stress in other plant species, while the COR413 and smGTP genes have less well-defined expression patterns as they relate to salt stress.

In order to determine the kinetics for salt-induced activation of these genes, a gel blot analysis of their transcript levels was performed in plants exposed to 500 mM NaCl over a 24 h period. As shown in Figure 4A, the smGTP and COR413 genes displayed low-level constitutive expression prior to salt stress (0 h). In untreated control plants, this low-level constitutive expression for the smGTP and COR413 genes remained constant over the 24 h time course (data not shown). All genes showed low induction after 2 h of treatment. The COR413 gene showed maximal activation after 8 h, with a slight reduction in transcript levels at 12 and 16 h, diminishing significantly after 24 h of stress treatment. The smGTP gene gradually increased after 4 h, reached its highest level after 16 h and displayed a slight reduction after 24 h of treatment. The P5CS transcripts were increased significantly by 4 h and this level was maintained up to 12 h followed by increases at 16 and 24 h of stress treatment.

To investigate the sensitivity of the P5CS, COR413 and smGTP genes to different levels of salt stress, 8-week-old mature plants were treated with increasing concentrations of NaCl and the gene activation was assessed by RNA blot analysis. As shown in Figure 4B, the COR413 gene showed the...
greatest sensitivity, displaying significant transcript accumulation after only 12 h exposure to 100 mM NaCl salt stress. The COR413 gene showed increased levels of expression with increasing NaCl concentration up to 300 mM, but then only minor increases in gene expression were observed when concentrations were increased to 400 and 500 mM NaCl. COR413 gene expression levels were increased after 12 h of salt exposure but then decreased after 24 h consistently at all NaCl concentrations tested. In contrast, the smGTP gene showed no significant activation until 300 mM NaCl salt stress was applied to the plants, with gradually increasing expression levels with increasing NaCl concentrations. The P5CS gene showed clear NaCl concentration-dependent transcript accumulation, with low-level induction observed at 100 and 200 mM NaCl concentrations, and maximal expression at 500 mM NaCl salt stress.

Effect of different abiotic stresses on gene expression

We examined the expression patterns of P5CS, COR413 and smGTP genes in response to various abiotic stress conditions. In Figure 5, as shown in the previous figures, all the 3 genes were activated by treatment with 500 mM NaCl. Surprisingly, PEG treatments resulted in activation of the COR413 gene in a pattern similar to that observed with salt stress, high levels of expression after 12 h of treatment that were decreased after 24 h. In contrast, the P5CS gene displayed a very low level of induction after 24 h and the smGTP showed no perceptible induction in response to the PEG-induced osmotic stress. It should be noted that symptoms of osmotic stress, such as loss in leaf stature and the initiation of leaf curling in the foliage of tillers, were clearly visible in treated...
plants 12 h after the PEG treatment. In well-hydrated plants subjected to constant heat stress (40°C), the COR413 gene was activated after 8 h of exposure while the smGTP and P5CS genes showed no induction. Similarly, when plants were subjected to a 5 min exposure to shortwave ultraviolet light, the COR413 gene displayed rapid induction 1 h later and a strong induction 8 h after the exposure to the UV light; however, neither the smGTP or the P5CS gene was induced. The COR413 gene was the only gene to be induced by mechanical wounding, while all the 3 genes were induced after 12 h of drought and after 24 h of cold treatment (4°C). Not surprisingly, the cold responsive gene COR413 showed its highest level of induction in response to the cold treatment.

SmGTP expression during seed development

During seed head development, tissues show signs of desiccation during different developmental stages; flowers wilt and senesce, seeds dry out and harden. Furthermore, during seed production, grasses in the field are cut while still somewhat green and physiologically active. The cut grass is allowed to cure in the fields for 1–2 weeks prior to harvesting the seed. During this time plant tissues are exposed to desiccation conditions and undergo senescence. To determine if the smGTP gene is involved in these processes, we investigated its expression levels during different stages of seed head development and in leaf and stem tissues after harvest. As shown in Figure 6A the smGTP gene had a low level of expression as the seed head emerged and showed a strong induction in late to post-anthesis tissues. Surprisingly very little expression was observed in seed heads at the milk and ripe seed head stages. In contrast to ripe seed heads, a low level of smGTP expression was observed in stem and leaf tissue at this stage of development. As shown in Figure 6B, the smGTP gene displayed low levels of expression prior to harvesting, increased 12 h after harvest, and appeared to remain at a constant level for the remainder of the experiment.

Discussion

Plants can utilize multiple signaling pathways and signals to mediate their response to various stresses; for example, at least 4 different signaling pathways have been identified for water deficit stress (Xiong et al., 2002; Chinnusamy et al., 2004; Munns, 2005; Yamaguchi-Shinozaki and Shinozaki, 2006). Different forms of stress may activate or utilize the same signaling components, including proteins and small molecules. One such class of signaling proteins is the GTPases. These signal-transducing polypeptides act as molecular on/off switches by hydrolyzing bound GTP to GDP. Analysis of a salt stress SSH expression library in the model grass species L. temulentum identified a gene encoding for a small GTP binding protein (smGTP). When the Lt-smGTP was compared to representative members of the Arabidopsis Rab family of small GTP binding proteins (Pereira-Leal and Seabra, 2001), it grouped with the RabB1b subfamily or Rab2-like proteins. While the plant Ras-related G proteins have been shown to be mobilized by abiotic stresses (Gu et al., 2004; Nibau et al., 2006), the Rab family of GTPases also have been shown to be involved with stress tolerance. A Rab5 GTPase family member was shown to be up-regulated in response to salt stress in the jojoba and ice plants (Bolte et al., 2000; Mizrahi-Aviv et al., 2002). In addition, the rice OsRab7 gene was also shown to be differentially regulated in response to cold, salt, dehydration and ABA (Nahm et al., 2003) and is grouped with the RabG subfamily. This subfamily also includes AtRab7, which was shown to confer salt and osmotic stress tolerance when over-expressed in Arabidopsis (Mazel et al., 2004).

Initial analysis of the smGTP gene showed that it had a low-level constitutive expression in root,
Salt stressed-induced Rab-like GTPase in Lolium temulentum

In L. temulentum, enzyme in proline biosynthesis. The genes were stress tolerance gene, COR413, SSH expression library, the cold response gene genes also identified in our analysis of a salt stress was then compared with two additional stress stresses. The expression pattern of the smGTP in order to transduce the signal in response to signaling protein would already need to be in place in order to transduce the signal in response to stresses. The expression pattern of the smGTP gene was then compared with two additional stress genes also identified in our analysis of a salt stress SSH expression library, the cold response gene COR413 and the well-characterized dehydration stress tolerance gene, P5CS encoding for a key enzyme in proline biosynthesis. The genes were analyzed for their response to salinity as well as their responses to 7 different forms of abiotic stress in L. temulentum plants.

When plants were subjected to increasing concentrations of salt stress, the COR413 gene was found to be more responsive to lower levels of salinity stress displaying a strong response at 100 mM NaCl concentrations. In contrast, the SmGTP and P5CS genes did not show a strong response until 300 mM NaCl. The COR413 gene was also found to be up-regulated in response to all forms of abiotic stress tested, whereas the P5CS and smGTP genes were only found to be strongly induced by salt, drought and cold stresses. The activation of smGTP and P5CS to both dehydration and cold stress is consistent with the current understanding of the transcriptional regulatory networks utilized by plants to survive these stresses (Yamaguchi-Shinozaki and Shinozaki, 2006). The results for the COR413 gene are similar to those found in Arabidopsis, wheat (Breton et al., 2003) and the resurrection plant (Garwe et al., 2003) where the gene was also generally found to be up-regulated to a variety of abiotic stresses. It is not surprising that this gene was induced by all forms of abiotic stresses tested, since most forms of abiotic stress affect membrane integrity and the COR413 gene encodes an integral membrane protein (Breton et al., 2003; Garwe et al., 2003). It is possible that the COR413 gene product may act to stabilize membranes during these stresses. Since the COR413 gene is up-regulated in response to all forms of abiotic stress, it has utility as a general stress marker in grass plants.

The P5CS gene encodes for a protein critical for stress tolerance which is involved in mediating the adverse effects of dehydration stress. The smGTP gene displayed an expression pattern similar to the P5CS gene, indicating that it may be involved in the regulation of water-related stress responses. Any temporal differences in gene expression between the P5CS and smGTP genes can be explained by their potentially different roles in mediating the plants response to salt stress. The P5CS gene is a well-characterized stress tolerance gene that is involved in the stabilization of the plant’s osmotic potential through the biosynthesis of proline. The smGTP gene has a different role in the stress response, potentially involved in signaling or vesicle transport. The induction of the smGTP gene under desiccating conditions during post-harvest stress and in post-anthesis seed heads, where flowers are showing signs of wilting as they begin to senesce, further support its role in dehydration stress. These results are similar to the induction of a closely related GTP-binding protein SsRab2 (O’Mahony and Oliver, 1999) during desiccation in the grass S. stapfianus (O’Mahony and Oliver, 1999).

The results described herein provide a valuable first step towards elucidating the role of this gene and its signaling cascade in grass stress responses. Currently very little is known about stress tolerance in grasses. Forage and turf grass species are generally polymorphic, obligate out-crossers that are self-incompatible. Because of their high genetic diversity, the mechanisms of salt and dehydration stress tolerance are poorly understood. It is becoming clear that despite the wealth of information and work in this field, there still remain many unknown components in the plant’s response to water-related stresses. Ultimately the identification of stress tolerance genes and their subsequent characterization in model grass species will lead to molecular and genetic approaches for forage and turf grass improvement.

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