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Differentially expressed genes induced by cold and UV-B in *Deschampsia antarctica* Desv.

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Abstract Antarctica is one of the most extreme environments on Earth. *Deschampsia antarctica* Desv. is the only monocot vascular plant that colonizes the Antarctic Peninsula. The survival of this species in this harsh environment suggests that this plant possesses genes associated with cold and UV tolerance. Using suppression subtractive hybridization, we identified a total of 112 differentially expressed genes under cold and UV irradiance conditions. Northern blot analysis and real-time RT-PCR confirmed expression differences among several genes. Using similarity search analysis, we identified a number of genes that have not been previously reported. The results showed that cold and UV radiation mainly induce the expression of genes related to transcription, energy and defense response. Interestingly, part of the isolated genes corresponds to unknown or hypothetical proteins. This set of tolerance-related genes could be relevant to uncover the mechanisms by which this extremophile survives in its environment and contribute to the development of biotechnology in Antarctic species.

Keywords Induced genes · Suppression subtractive hybridization · Cold and UV-B tolerance · Antarctica

Abbreviations

EST Expressed sequence tags
SSH Suppression subtractive hybridization
PFD Photon flux density

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Introduction

Plants are constantly exposed to environmental stresses, and their survival is determined by their adaptation to short- and long-term stresses (Nishizawa-Yokoi et al. 2011). Environmental stress affects crop growth and poses a threat for sustainable agriculture. This issue has become a relevant concern due to the effects of recent climate change on plant resources, biodiversity and global food security (Ahuja et al. 2010). The ability of wild plants to survive in extreme environments generates great interest, because of the physiological mechanisms and genetic regulation that control the adaptation of plants to environmental stress. A major part of plant adaptation to abiotic stresses is controlled at the level of gene expression (Vaahtera and

Brosche 2011). Genes whose expression is modified under stress conditions are often found to be important for adaptation to stress, and transgenic plants that overexpress such genes have shown increased stress tolerance (Hirayama and Shinozaki 2010).

Low temperature is a major factor limiting the productivity and geographical distribution of many species, including important agricultural crops (Allen and Ort 2001). Each plant has its unique set of temperature requirements, which are optimum for its proper growth and development (Mahajan and Tuteja 2005). Low temperature can damage the vegetative tissue by inducing osmotic, oxidative and other stresses (Thomashow 1999). Moreover, the combined exposure of plants to low temperature and high irradiance, which decrease carbon and other enzymatic assimilation processes, causes an imbalance in the energy utilization (Huner et al. 1998). In plant cells, extracellular freezing damages membrane systems. This damage is largely due to the acute dehydration associated with freezing (Mahajan and Tuteja 2005; Chen et al. 2011). Reactive oxygen species (ROS) that are produced in response to cold stress may contribute to membrane damage. Overall, cold stress results in loss of membrane integrity that leads to solute leakage (Mahajan and Tuteja 2005; Pál et al. 2011). The integrity of intracellular organelles is also disrupted, leading to the loss of compartmentalization, reduction in and impairment of photosynthesis, protein assembly and general metabolic processes (Mahajan and Tuteja 2005). Plants can diminish the deleterious effects of low temperature by means of cold acclimation. In this process, plants increase their cold tolerance in response to prior exposure to low non-freezing temperatures (Mahajan and Tuteja 2005). The acclimation is associated with the de novo expression of genes, protein synthesis and various physiological changes (Guy et al. 1985). The mechanisms that contribute to tolerance include the prevention of protein denaturation induced by low temperatures, preventing the precipitation of molecules and the attenuation of the effects of intracellular ice formation (Sung et al. 2003).

The UV-B irradiance (ultraviolet-B, 280–315 nm) reaching the Earth's surface has increased due to the ozone depletion in recent years (Huiskes et al. 1999). Recent reviews have discussed the direct and indirect effects of enhanced and ambient UV-B radiation on vascular plants and terrestrial ecosystems (Robinson et al. 2003). On a molecular level, UV-B can directly damage biomolecules such as DNA, RNA, proteins and lipids (Frohnmeyer and Staiger 2003). In DNA, cyclobutane–pyrimidine dimmers and pyrimidine–pyrimidine dimmers are formed during irradiation of plants with UV-B (Britt 1999). In contrast, low fluence rates of UV-B are sufficient to initiate regulatory responses. For example, low levels of UV-B inhibit stem extension, stimulate cotyledon opening and promote the accumulation of flavonoids (Kucera et al. 2003).

The harsh environment of the Antarctic Peninsula limits most life forms, and vascular plants in particular. The Antarctic hairgrass *Deschampsia antarctica* Desv. (Poaceae family) is the only monocot naturally adapted to Antarctic conditions (Xiong et al. 1999; Bravo et al. 2001). Among the characteristics that may be implicated in the survival of this Poaceae in the adverse Antarctic environment are the following: tolerance to extracellular ice, a photosynthetic apparatus that maintains 30 % of the optimal photosynthetic rate at 0 °C, the accumulation of carbohydrates such as fructans and sucrose, and a high level of flavonoids (Xiong et al. 1999; Bravo et al. 2001; Alberdi et al. 2002; Ruhland et al. 2005; Pereira et al. 2009). *D. antarctica* Desv. growing season is limited to the Antarctic summer, when the average night and day temperatures are −2 and 6 °C, respectively. It has been shown that *D. antarctica* Desv. is an unusual species that expresses apoplastic antifreeze proteins constitutively (Bravo and Griffith 2005). Gidekel et al. (2003) verified the de novo synthesis of proteins by means of 2D PAGE, showing qualitative and quantitative differences between cold-acclimated and non-acclimated plants. In addition, they isolated specifically transcribed genes induced by low temperature stress by means of differential display. Lee et al. (2008) identified responsive genes in *D. antarctica* Desv. by means of EST analysis, comparing cDNA libraries from greenhouse plants with plants growing in Antarctica.

Suppression subtractive hybridization (SSH) allows the construction of small cDNA libraries enriched with differentially expressed transcripts that are present only in one of the compared samples (Diatchenko et al. 1996). This method has been used efficiently in several organisms to identify genes that are induced by pathogenic infection in plants and by different biotic and abiotic stresses (Sanchez-Ballesta et al. 2003).

D. antarctica Desv. is a extremophile plant surviving in one of the harshest climates on Earth. Its ability to adapt and tolerate the extreme environmental conditions allows it to colonize the Antarctic Peninsula (Alberdi et al. 2002). Thus, *D. antarctica* Desv. represents a potentially invaluable source of genes associated with tolerance to abiotic stresses. In this study, we set out to identify new genes that could be involved in *D. antarctica*'s tolerance to cold and UV-B. We have exposed plants to different temperature and UV irradiation conditions, and by means of SSH, we identified a set of differentially expressed genes. Sequence alignment and gene expression analysis revealed new genes that have not been reported previously as tolerance-responsive genes. In addition, we have found several ESTs coding for unknown proteins. The importance of some of the identified genes for the mechanisms of tolerance to cold and UV radiation will be discussed.

Materials and methods

Plant material and growth conditions

Deschampsiaantarctica plants were collected in the Collins glacier on King George Island, maritime Antarctic (62°10′05, 6″S; 58°51′06, 2″W) in the summer of 2008. The photoperiod at that time of year was close to 21/3 h light/dark, and the average daily temperature is 6 °C. The sampling site was about 20 m from the seashore, in close proximity to an area frequented by Antarctic birds and animals, enriched with nutrients and subjected to sea spray. At this location, *D. antarctica* grow forming a very dense homogeneous lawn-like population.

The effect of UV-B was studied in the field comparing naturally growing plants with an experimental group in which the UV-B was filtered using Mylar®D (Dupont Corp). Filters were placed on a wooden frame (100 cm long, 80 cm wide and 65 cm height) over naturally growing plants for a period of 21 days. The frame was open on two sides to allow air circulation and to prevent overheating effect. Soil and canopy temperature were monitored with data loggers using shielded fine-wire copper-constantan thermocouples. We did not find any significant difference between the plants under the frame and those at the open field (data not shown). There was no register of rain or snow during the experiment. Fresh tissue (5 g approximately) was collected between 10:00 am–2:00 pm (maximum period of UV-B radiation). Samples were frozen immediately in liquid nitrogen and then stored at −80 °C until used for RNA isolation.

A set of untreated plants from the same area, at the same developmental stage, were placed in plastic containers and transported to Chile. In the laboratory, plants were cultivated in plastic pots using a peat/soil mixture (3:1) and maintained at 13 ± 1.5 °C (optimum growth temperature) (Edwards and Lewis Smith 1988) in a growth chamber with a photon flux density (PFD) of 100–120 μmol photons m^{-2} s^{-1} at the top of the canopy and a 21-/3-h light/dark photoperiod. The light source consisted of cool-white fluorescent tubes F40CW (General Electric, USA). This irradiance corresponds to saturating PFD in laboratory-grown Antarctic grass plants (Edwards and Lewis Smith 1988).

As described by other groups (Gielwanowska and Szczuka 2005), we observed morphological changes in plants growing in laboratory conditions for long periods of time. To minimize differences due to these morphological changes, we sampled the tissue from plants growing in laboratory conditions for a period of 3–4 months. These plants showed similar morphology to those growing under natural conditions.

RNA isolation

Total RNA of whole plants was extracted from three independent biological replicates using Trizol reagent (Invitrogen, USA), according to the manufacturer's protocol, and treated with RNase-free DNase (Promega, USA). The treatment was followed by total RNA clean up using RNeasy mini spin columns (Qiagen, USA). RNA concentration was determined spectrophotometrically using a ND-1000 spectrophotometer (NanoDrop Technologies, USA), and its integrity was assessed by agarose/ formaldehyde gel electrophoresis. Poly(A) + RNA was purified from total RNA using the Dynabeads mRNA Purification Kit (Dynal Biotech Inc., USA) according to the manufacturer's protocol.

Suppression subtractive hybridization

Two differentially expressed gene libraries were obtained by means of SSH. For the ANT vs LAB experiment, the SSH was carried out between Antarctic-grown (tester 1) and laboratory-grown (driver 1) plants. For the UV vs non-UV library, the UV-treated (tester 2) and non-UV (driver 2) plants were used. The SSH procedure was performed with the PCR-Select cDNA Subtraction Kit (BD Biosciences-Clontech, USA) according to the manufacturer's instructions. All PCRs during the SSH procedure were performed in a Hybaid thermal cycler (Hybaid Ltd., Middlesex, UK). The PCR mixture enriched for differentially expressed sequences from the tester populations were cloned using the pGEMT-easy cloning kit (Promega, USA) and $pCR_{2.1}$TOPO (Invitrogen, USA) for the ANT/LAB and UV/non-UV libraries, respectively, according to manufacturer's instructions, and transformed into DH5α *Escherichia coli* cells for blue–white selection. The subtracted libraries contained approximately 1,700 clones for the ANT/LAB library and 2,000 clones for the UV/non-UV library. Clones were primarily screened by means of a cDNA array approach (PCR-Select Differential Screening kit, Clontech, USA) according to the manufacturer's recommendations.

Sequencing and sequence analysis

A set of clones from each library was selected, and plasmid DNA was purified from each clone. Cycle sequencing reactions were prepared and run in an AbiPrism 3700 sequencer (Applied Biosystems, USA). Each sequence was edited to correct sequencing ambiguities and to remove the primer and vector sequences. The edited sequences were analyzed by sequence similarity searches against the NCBI nr database, using the BLASTX algorithm with a cutoff e value of 10E−6 (Altschul et al. 1990) to assign predicted

functions. The generated EST sequences were deposited into GenBank with accession numbers from FG548273 to FG548386.

RNA blot analysis

Total RNA (5 µg) was separated by agarose/ formaldehyde gel electrophoresis and transferred to Hybond N+ nylon membrane (Amersham-Pharmacia, UK). Probes were made from PCR-amplified fragments of selected clones using the Hexalabel DNA Labeling Kit (Fermentas, USA) with α-32P. Blots were hybridized and washed according to standard procedures (Lee et al. 1992).

cDNA synthesis and quantitative real-time PCR

Three independent cDNA synthesis reactions (three biological replicates) were conducted for each condition ((A) ANT/LAB library and (B) UV/ non-UV Library). 1 µg of DNA-free total RNA was reverse-transcribed using the Affinity Script qRT-PCR cDNA Synthesis Kit (Stratagene, USA). The cDNA was quantified using a master mix with an oligonucleotide primer to identify the sequences and the Brilliant II SYBR Green qRT-PCR Master Mix (Stratagene, USA), following supplier's instructions. The reactions were performed in a M×3000 real-time PCR system (Stratagene, USA), including non-RT controls and non-template controls. The expression level of five reference genes (α-TUB, UBQ5, rRNA18S, ACT1 and GAPDH) was assessed in samples the *D. antarctica* Desv. exposed to cold and UV-B stress conditions. These genes were selected because of their common use as references in qPCR analysis. Gene stability was analyzed by the geNorm software (http://medgen.ugent.be/~jvdesomp/genorm/).

The primer sets were designed by using AmplifX 1.5.4 software. The name of the gene, symbol, forward primer and reverse primer used for qPCR are shown in Table 1. The PCR was preceded by incubation at 95 °C for 10 min; then, the samples underwent 40 cycles of 95 °C for 15 s, 60 °C for 15 s and 72 °C for 15 s followed by extension at 72 °C for 10 s.

A *t* test statistic analysis was performed to observe significant differences between the measurements.

Results

Analysis of SSH cDNA *library*

Plants of *Deschampsia antarctica* Desv. growing under the specified treatments (See Materials and Methods) were used to construct the SSH libraries. The libraries contain both normalized and enriched differentially expressed genes. Dot-blot analysis of 850 PCR products from randomly selected clones from these subtracted libraries showed that 261 clones might correspond to differentially expressed genes (data not shown). After screening, a total of 112 EST sequences were obtained and analyzed. Putative homologues were identified on the basis of systematic comparison with sequence databases. Through sequence analysis, we were able to identify or assign putative functions to about half (52) of the genes (E values $<10^{-3}$). The clones corresponding to proteins with insufficient similarity (E values $\geq 10^{-3}$) were classified as being of unknown function or hypothetical proteins. The sequences that matched known genes were sorted into 12 primary functional categories (Bevan et al. 1998). The distribution of the genes with known functions is represented as a pie chart in Fig. 1. The largest set of genes (30–40 %) in both libraries was assigned to the Energy category. EST sequences were submitted to GenBank with the following accession numbers (FG548273–FG548386) (Table 2).

Validation of selected genes expression

The SSH libraries were validated by assessing the expression level of a set of 8 differentially expressed ESTs selected from the libraries using Northern blot and qRT-PCR (Figs. 2, 3, respectively). The expression stability of the five reference genes (α-TUB, UBQ5, rRNA18S, ACT1 and GAPDH) was analyzed using the geNorm software. A low M (average expression stability) value (<1.5) is indicative of a more stable expression, hence, increasing the suitability of a particular gene as a control gene. We found that the most stable reference genes differed depending on the stress condition. α-TUB showed the most stable expression in both conditions, cold and UV-B (1.06). The PCR efficiency of all genes ranged from 96 to 100 % in the different conditions (data not shown). The results showed that transcripts evaluated increased their relative expression levels after cold or UV-B treatment (Fig. 3), thus validating SSH technique results.

Discussion

Plants are regularly exposed to various environmental stresses such as low or high temperatures, dehydration, high salinity, infection and injuries. In the course of evolution, many protective mechanisms have been developed by plant cells to overcome environmental stresses. To get an insight into the molecular mechanisms associated with cold and UV-B tolerance, we constructed two SSH libraries enriched for differentially expressed genes in *D. antarctica* Desv. plants grown under ANT/LAB and UV/non-UV conditions. One of the main advantages of SSH is that it normalizes the

Table 1 Genes and primer pairs used in the qRT-PCR analysis

Name	Symbol	Description		Primer 5′–3′
Daα-TUB	α-TUB	α-TUB	Fw	AGCTGAGAAGGCTTACCATGAGCA[a]
			Rv	ACTGGATAGTGCGCTTGGTCTTGA[a]
Da5F11	G3PD	Glyceraldehyde 3-phosphate dehydrogenase	Fw	AGCCTTTTGGCCCTGTCTTG
			Rv	TGACGGTGCTCTTCACCTTG
Da9G8	RuBisCO	Ribulose 1,5-bisphosphate carboxylase large subunit	Fw	GGGGATTCACCGCAAATACT
			Rv	CCCAGACATACGCAATGCTT
Da11H13	FtsH	FtsH-like protein	Fw	ACAAGAAGTTTGATCCCGATG
			Rv	CATGCCAGCCACTATTCTG
Da3D11	UnKn1	Unknown protein	Fw	GACCGTGTCAAGAAAGTAGAG
			Rv	ATGCATCTGAGGTCTGAGG
Da6D11	CAT	Catalase	Fw	GATTTGCTCGCACAATTGAC
			Rv	CATCAACAGATGGATCGACG
Da1D8	Chap	Chaperone protein	Fw	AGAAGGTGCAGAAGGAGTGT
			Rv	GTTGGAAACGCCTTCCTTGA
Da5H3	CA	Carbonic anhydrase	Fw	GCATCTACCCACTGTGATCTTG
			Rv	ACAGCACCATGCAGAAACC
Da1E12	UnKn2	Unknown protein	Fw	CCGCCTGATTACGAGTTACA
			Rv	GTTATCTCCCGCTAACAGCA
DaSOD	SOD	Superoxide dismutase	Fw	ATGGTGAAGGCTGTAGCTGTGC
			Rv	TCTTCTGGTGCCCCATGCA

[a] Lee et al. (2008)

cDNA abundance so that cDNAs encoded by genes that are expressed infrequently, but none the less differentially, can be readily identified (Diatchenko et al. 1996).

Non-redundant consensus sequences with inserts from 100 to 800 bp were analyzed using BLASTX algorithm and compared to the NCBI non-redundant protein database. Sequence analysis of 112 (75 from ANT/LAB and 37 from UV/non-UV) non-redundant cDNAs (Accession numbers FG548273–FG548386) revealed that 52 (45 %) had significant similarity to genes encoding proteins with a known function (Table 2). In addition, 60 clones either encoded proteins with insufficient similarity to proteins of known function or were identified as hypothetical proteins. An unusually high percentage of the selected genes, 63 % (47) of the ANT/LAB and 34 % (13) of the UV/non-UV libraries, corresponded to unknown or hypothetical genes (data not shown). This group of genes with unknown function could represent a new set of genes to study as they may be important for the survival of *D. antarctica* Desv. under the harsh conditions of the Antarctic peninsula.

The genes with identified functions were sorted into 12 primary functional categories (Fig. 1) (Bevan et al. 1998). The largest set of genes in both libraries was assigned to "Energy" category, followed by "Signal transduction" and "Metabolism" in the UV/non-UV library, and "Transcription" and "Protein destination and storage" in the ANT/LAB library.

The differential nature of the SSH libraries was confirmed by Northern blot of selected genes (Fig. 2). In addition, a group of genes from each SSH library was selected and used as targets for qRT-PCR differential expression analysis. The selected genes were differentially expressed (Fig. 3a, b). The expression of tested genes was higher in the Antarctic plants than in plants grown under laboratory or non-UV conditions.

Environmental factors are known to influence gene expression. The group related to Energy includes 17 distinct transcripts, and in both libraries, RuBisCO (*RbcL*) is induced (Fig. 1; Table 2). This result differs from previous reports on other Poaceae, where RuBisCO was downregulated by UV-B radiation (Casati and Walbot 2003). Hashimoto and Komatsu (2007) identified cold stress-responsive proteins in rice leaf blades, among which the *rbc*L gene was upregulated, suggesting an increase in the transcript abundance of RuBisCO. Importantly, most of "Energy" identified genes participate in carbohydrates production. Accumulation of carbohydrates and solutes has been suggested as a protective mechanism against low temperature (Zúñiga-Feest et al. 2005).

Numerous genes inducible by low temperatures and their promoters have been isolated and characterized in several plant species including *D. antarctica* Desv. (Gidekel et al. 2003). Genes induced by low temperature and UV-B radiation encode proteins with signaling functions, signal transduction and cell protection, such as protein constituents of water channels that alter cellular water potential (Taj et al. 2010), enzymes required for the synthesis of protective substances (sugars, proline and betaine) (Kumar and Yadav 2009), lipid desaturases to modify the

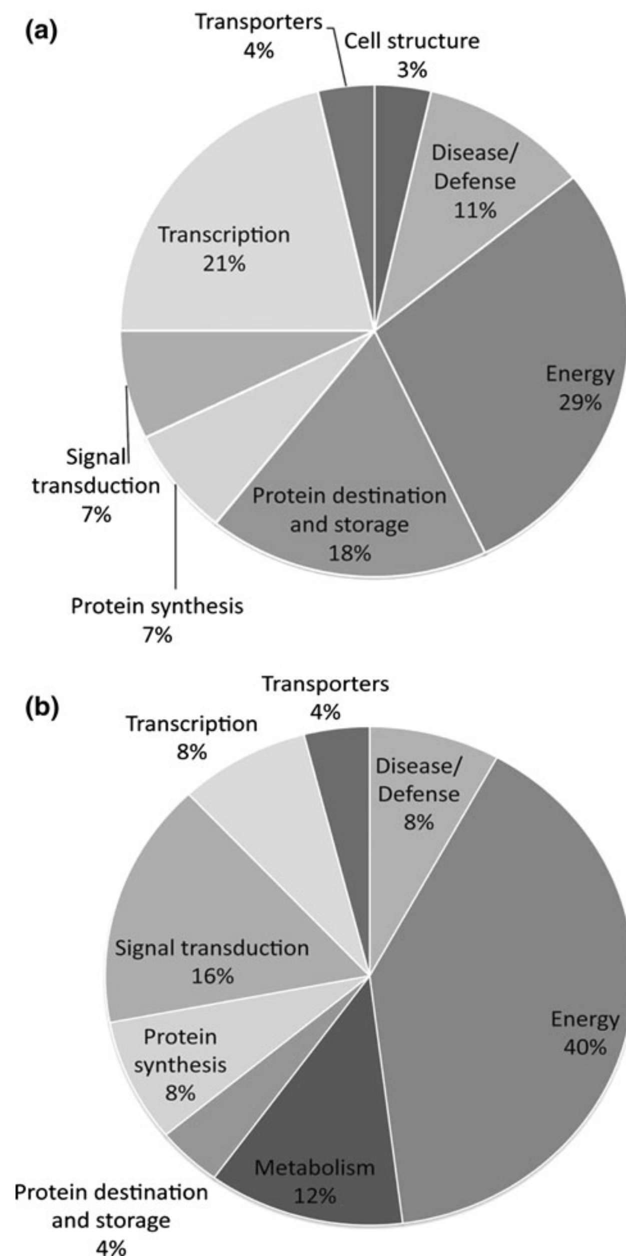

Fig. 1 Potential regulatory genes. *Pie charts* showing the fraction of identified genes from each library. The cDNAs sequences were analyzed by similarity search, and the genes were grouped according to their predicted function. **a** ANT/LAB Library, **b** UV/non-UV Library

composition of cell membranes (De Palma et al. 2008), protective proteins such as those of LEA (Late Embryogenesis Abundant), antifreeze proteins, chaperones, proteases and detoxifying enzymes such as catalase and ascorbate peroxidase (Amudha and Balasubramani 2011). The proteins encoded by these genes contribute to abiotic stress tolerance.

One of the most important genes induced in the ANT/LAB library was the transcriptional factor CBF1 (Table 2). Currently, the best documented genetic pathway leading to gene induction under low temperature conditions is mediated by the Arabidopsis C-repeat/dehydration-responsive element binding factors (CBFs), a small family of three transcriptional activators (CBF1-3) that bind to the C-repeat/dehydration-responsive elements (Medina et al. 2011). The CBF/DREB1 proteins have been identified that control the expression of a regulon of cold-induced genes that increase plant freezing tolerance (Thomashow 1999). Overexpression of *CBF1/DREB1b* and *CBF3/DREB1a* enhances cold tolerance by inducing *COR* (cold-regulated) genes (Gilmour et al. 2000). Furthermore, its overexpression causes many biochemical changes, such as accumulation of sugar and proline. Thus, the *CBF/DREB1* genes are believed to be the activators that integrate several components of the cold acclimation response, by which plants increase their tolerance to low temperature after exposure to non-freezing conditions (Gilmour et al. 2000).

Glyceraldehyde-3-phosphate dehydrogenase (*G3PDH*) was upregulated in Antarctic conditions. The genetic expression of *G3PDH*, a key enzyme of the glycolytic pathway, has been shown to increase under environmental stress conditions in *Zea mays* (Russell and Sachs 1989), *Arabidopsis thaliana* (Yang et al. 1993) and apple (Ban et al. 2007), including UV irradiation and acclimation to cold. *G3PDH* regulates the response of cells to salt stress and may aid in the development of new salt-tolerant cultivars in soybean (Xu et al. 2011). When *Dunaliella salina*, an extremely halotolerant, green microalgae, was treated by hyperosmotic or hypoosmotic shock, the activity of *G3PDH* expressed significant correlation with salinity, suggesting that the whole metabolic pathway of glycerol was driven by *G3PDH* when *D. salina* encountered osmotic stresses. (Chen et al. 2012).

Protein degradation within the cell plays important roles, either by modulating intracellular levels of specific proteins such as by removal of aberrant proteins. Currently, it is also known that the degradation of certain proteins is the control point of diverse biological processes, some as fundamental as cell cycle progression. The FtsH-like protein Pftf precursor (FtsH) was induced in Antarctic plants. It is known that FtsH is a component of the complex PSII repair system that operates to overcome photoinhibition in plants (Lindahl et al. 2000). FtsH protease is also required for efficient repair of UV-damaged PSII centers (Cheregi et al. 2005).

The UV-B irradiance that induces diverse morphological and physiological responses in plants (Paul and Gwynn-Jones 2003) can also cause macromolecular damage and inhibit cellular processes by damaging biomolecules directly and by increasing the production of oxygen reactive species (ROS). In response to the inevitable exposure to UV-B radiation damage, plants have evolved

Table 2 Identification and functional classification of genes *Deschampsia antarctica* Desv.

Clone[a]	Accession number	Putative identification[b]	Functional category
DaANT-12E5	FG548294	Possible integral membrane	Cell structure
DaANT-6A11	FG548282	Acclimation protein	Disease/defense
DaANT-11F4	FG548285	Enolase	Energy
DaANT-12D2	FG548336	Ferredoxin-NADP(H) oxidoreductase	Energy
DaANT-3F6	FG548333	Putative malate dehydrogenase	Energy
DaANT-5F11	FG548315	Glyceraldehyde-3-phosphate dehydrogenase	Energy
DaANT-6B6	FG548295	Glyceraldehyde 3-phosphate dehydrogenase	Energy
DaANT-6D10	FG548284	Phosphoribulokinase	Energy
DaANT-6E7	FG548337	RuBisCO large subunit	Energy
DaANT-7H11	FG548312	NADH dehydrogenase subunit 4	Energy
DaANT-9G8	FG548289	RuBisCO large subunit	Energy
DaANT-6H8	FG548299	Glyoxalase I	Metabolism
DaANT-7H7	FG548304	Cytosolic monodehydroascorbate reductase	Metabolism
DaANT-11B8	FG548324	Proteasome subunit	Protein destination and storage
DaANT-11C7	FG548293	Uba1 gene product-related.	Protein destination and storage
DaANT-11H3	FG548286	FtsH-like protein Pftf precursor	Protein destination and storage
DaANT-7C9	FG548338	Cysteine proteinase	Protein destination and storage
DaANT-11B3	FG548305	Ribosomal protein S7	Protein synthesis
DaANT-11G7	FG548327	Putative protein translation factor Sui1	Protein synthesis
DaANT-9A3	FG548323	Putative inositol 1,3,4-trisphosphate 5/6-kinase	Signal transduction
DaANT-11C4	FG548342	Maturase K	Transcription
DaANT-1B6	FG548273	Transcriptional activator CBF1	Transcription
DaANT-2B8	FG548320	Putative root hair defective 3	Transcription
DaANT-4D8	FG548334	Cleavage and polyadenylation-specific factor	Transcription
DaANT-4E1	FG548341	Cold-regulated protein BLT14	Transcription
DaANT-6G2	FG548347	SCARECROW-like protein	Transcription
DaANT-7G7	FG548345	Homeobox transcription factor	Transcription
DaANT-4E9	FG548278	Putative Ca2+/H+-exchanging protein	Transporters
DaUV-2F4	FG548360	Beta-1,3-glucanase	Defense
DaUV-2A3	FG548364	Metallothionein-like protein type 3	Disease/defense
DaUV-6D11	FG548375	Catalase	Disease/defense
DaUV-10E10	FG548371	RuBisCO, RBS2	Energy
DaUV-13D1	FG548368	RuBisCO, large subunit	Energy
DaUV-13G10	FG548356	RuBisCO, RBS3	Energy
DaUV-15A7	FG548362	RuBisCO, RBS1	Energy
DaUV-2A9	FG548373	LHC chlorophyll a/b-binding protein (Cab4)	Energy
DaUV-2D9	FG548365	Chlorophyll a/b-binding protein CP29	Energy
DaUV-2E12	FG548352	Photosystem I subunit psaN	Energy
DaUV-2F9	FG548366	Chlorophyll a/b-binding protein type I	Energy
DaUV-5H3	FG548367	Carbonic anhydrase	Energy
DaUV-1A12	FG548349	Acyl-CoA synthetase	Metabolism
DaUV-1C5	FG548372	Aspartate kinase	Metabolism
DaUV-1D3	FG548380	Cellulase	Metabolism
DaUV-1D8	FG548384	Chaperone protein	Protein destination and storage
DaUV-11E4	FG548376	60S ribosomal protein L13A	Protein synthesis
DaUV-1E6	FG548350	Cytosolic ribosomal protein S11	Protein synthesis
DaUV-1C12	FG548377	Shaggy-like kinase alpha	Signal transduction
DaUV-3H4	FG548353	Phosphoinositide-specific phospholipase C	Signal transduction

Table 2 continued

Clone[a]	Accession number	Putative identification[b]	Functional category
DaUV-7D2	FG548386	CBL-interacting protein kinase	Signal transduction
DaUV-1B10	FG548363	TatC	Transcription
DaUV-2C12	FG548359	Kruppel-like factor 1	Transcription
DaUV-2H3	FG548370	Transporter-related protein	Transporters

[a] DaANT and DaUV indicate clones belonging to ANT/LAB and UV/non-UV, respectively

[b] Identification based on sequence similarity search (BLASTX)

UV-induced protection and repair mechanisms such as accumulation of UV-absorbing pigments (Bieza and Lois 2001), the use of UV-A photons to repair most UV-B-induced DNA damage (Britt 1996) and defense systems against oxidative damage (Scandalios 2005).

Another important gene found to be overexpressed is a molecular chaperone. These groups of proteins are key components that contribute to cellular homeostasis in cells under both optimal and adverse growth conditions. They are responsible for protein folding, assembly, translocation and degradation in a broad array of normal cellular processes; they also play a role in the stabilization of proteins and membranes and can assist in protein refolding under stress conditions (Wang et al. 2004). Many molecular chaperones are stress proteins (Wang et al. 2004). Chaperones are known to be expressed in plants not only when they experience high temperature stress (Hsps/Chap) but also in response to a wide range of other environmental insults such as water, salinity and osmotic, cold and oxidative stresses (Wang et al. 2004).

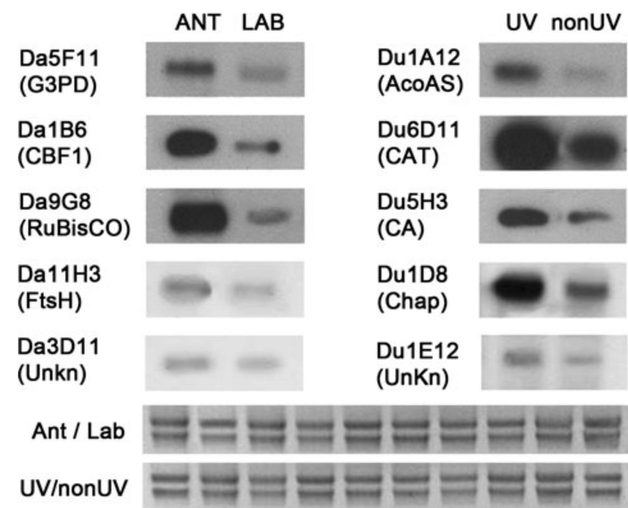

Fig. 2 Representative Northern blot analysis of stress-modulated genes using cloned cDNA fragments from the SSH libraries. Total RNA was extracted from plants under ANT/LAB (*left*) and UV/non-UV (*right*) conditions. The putatively identified gene names are shown in *parentheses*; *G3PD* glyceraldehyde 3-phosphate dehydrogenase, *CBF1* transcriptional activator CBF1, *RuBisCO* Ribulose 1,5-bisphosphate carboxylase large subunit, *FtsH* FtsH-like protein, *AcoAS* Acyl-CoA synthetase, *CAT* catalase, *CA* carbonic anhydrase, *Chap* chaperone protein, *UnKn* unknown protein. *Lower panels* show portions of the ethidium bromide stained gels used for Northern blotting to indicate the equal loading of RNA samples

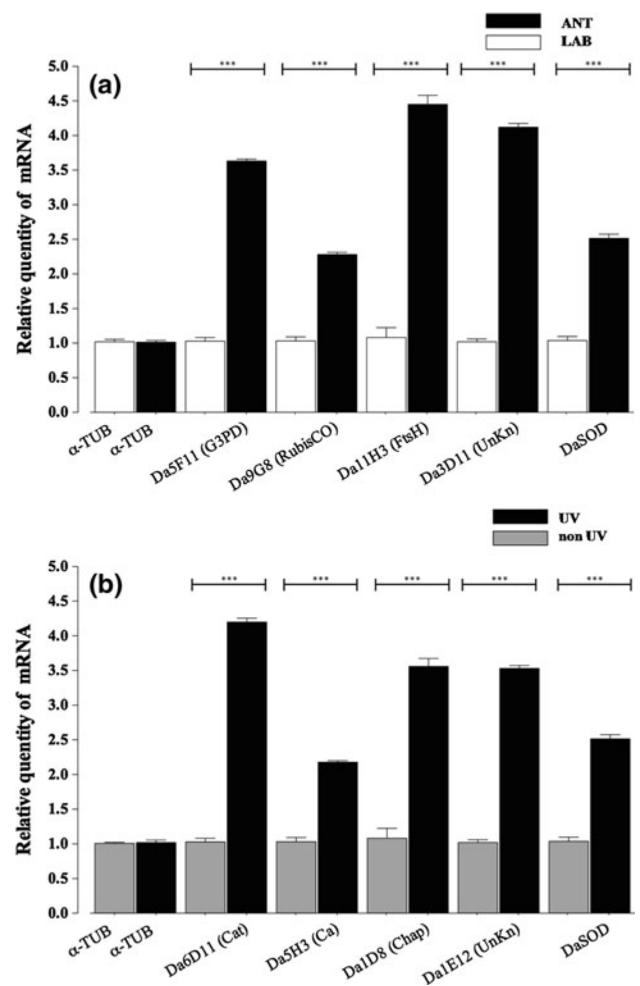

Fig. 3 Gene expression analysis. The expression of a group of selected genes was determined by real-time PCR. Relative levels were calculated by comparing gene expression between Antarctic plants (*black bars*) and **a** laboratory plants (*white bars*) and **b** non-UV plants (*gray bars*) after normalization with the α-TUB gene. The data are shown after normalization to the expression of α-TUB. The values represent the mean ± SD of three independent experiments. Significantly different from control, *** < 0.001 (Student's *t* test for paired data)

We found that genes encoding antioxidative enzymes catalase and superoxide dismutase (control) were induced upon UV-B exposure (Fig. 3), supporting their significant stress tolerance role. In plants, the most frequent effect of exposure to elevated UV radiation is oxidative stress and ROS overproduction, in which a significant role is played by hydrogen peroxide. Superoxide dismutase generates H_2O_2, and catalase is one of the main enzymes that scavenge hydrogen peroxide (Rybus-Zając and Kubiś 2010). This group of enzymes is highly active in *D. antarctica* Desv. and it has been suggested that they may play an important role in the survival of this species (Pérez-Torres et al. 2004). UV-B also induced the expression of a carbonic anhydrase (CA). The expression of this gene was found to be enhanced in plants in response to salt treatment (Yu et al. 2007).

In summary, we have identified and isolated 112 cold and UV-B (75 and 37, respectively)-induced genes from *D. antarctica* Desv. Furthermore, 52 of these genes encode known proteins, whereas 62 of them correspond to hypothetical proteins. This group may contain tolerance candidate genes; therefore, their characterization may lead to a better understanding of the tolerance mechanisms in this and in other plant species, and for future use in the crop improvement. Enriched cDNA libraries, such as the one we have constructed, may contribute to the characterization of the stress transcriptome through the construction of specialized arrays. Future attempts to identify the unknown genes may provide us with better understanding of their role in cold and UV-tolerance mechanism.

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