

# Transcription factor profile analysis of the Antarctic vascular plant *Deschampsia antarctica* Desv. (Poaceae)

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**Abstract** Transcription factors (TFs), which control gene expression through sequence-specific interactions with *cis*-elements of downstream gene promoters, play an important role in developmental processes and in response to environmental stress. To explore the molecular mechanism behind stress signaling and the development of Antarctic hairgrass (*Deschampsia antarctica* Desv.), a terrestrial plant that has successfully adapted to the Antarctic climate, we analyzed the *D. antarctica* EST database and constructed a TF profile based on sequence homology searches with other plant TFs to identify 1,083 transcripts encoding TFs categorized into 53 TF families. The gene ontology distribution of TF-encoding transcripts and lineage-specific expansion/contraction of TF families were analyzed. In addition, we identified a group of putative abiotic stress-induced TFs by comparing EST libraries generated under different abiotic conditions and validated the results using quantitative real-time PCR. Since plant TFs are primary targets for genetic engineering and the development of stress-tolerant crops, these results could be a useful resource for agricultural applications.

**Keywords** Abiotic stress · Antarctic hairgrass · Poaceae · Transcription factor

## Introduction

Transcription factors (TFs) are DNA-binding proteins that regulate gene expression by recruiting transcriptional

machinery to gene promoters or blocking their access. The specific interaction between TF proteins and the *cis*-regulatory sequences in downstream genes plays a central role in developmental processes and in response to environmental changes. TF genes account for a considerable amount of eukaryotic genomes (~7 % in plants) and are represented by multigene families categorized according to the type of DNA-binding domain they encode (Riechmann et al. 2000; Libault et al. 2009). The TF family is highly conserved in eukaryotes, especially in the plant kingdom. However, the number of genes for a particular TF family vary in different plant species with expansion or contraction of specific TF families, which might occur during large-scale genome duplication events in their evolutionary history (Lespinet et al. 2002; Shiu et al. 2005; Libault et al. 2009; Garg et al. 2011). Hence, the identification and characterization of TF repertoire from a specific species may expand our understanding of the evolution of TF genetic networks.

As sessile organisms, higher plants have evolved stress-tolerance mechanisms to endure environmental challenges such as soil salinity, drought, and cold temperatures, which influence the development of plants and threatens the productivity of crops worldwide (Thomashow 1999; Zhu 2001). Stress acclimation and the acquisition of stress tolerance require an orchestrated mechanism regulated by multiple signaling pathways that activate gene transcription and its downstream cascade. Previous studies have identified key regulators responsible for stress tolerance and have elucidated the transcriptional network between the key regulators and downstream stress-responsive genes. In plants, several families of TFs have been implicated in plant stress responses. For example, the DREB1/CBF family is a member of AP2/ERF-type TFs, which recognizes cold-responsive *cis*-elements and DRE/CRT. Overexpression of these genes results in enhanced

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tolerance to drought, salt, and freezing (Jaglo-Ottosen et al. 1998; Kasuga et al. 1999). INDUCER OF CBF EXPRESSION 1 (ICE1), a MYC TF and the calmodulin-binding transcription activator (CAMTA) are *trans*-acting regulators of DREB1A/CBF3 and DREB1C/CBF2, respectively (Chinnusamy et al. 2003; Doherty et al. 2009). ZAT12, a zinc finger protein, is regulated by CAMTA and downregulates the expression of DREB/CBF genes (Vogel et al. 2005). In *Arabidopsis* and grasses, while the amount of DREB1 genes rapidly increases at cold temperatures, DREB2 and other AP2-type TFs that recognize DRE/CRT are involved in dehydration or salinity-stress responses (Liu et al. 1998; Nakashima et al. 2000). Osmotic stress by drought and high salinity increases abscisic acid (ABA) levels; this in turn induces ABA-responsive TFs (AREB/ABF) containing the bZIP DNA-binding motif, which binds to a conserved ABA-responsive element designated ABRE (PyACGTGG/TC), a major *cis*-element in ABA-responsive gene promoters (Leung and Giraudat 1998; Shinozaki and Yamaguchi-Shinozaki 2000). Osmotic stress activates several other TFs, including NAC and zinc finger homeodomain (ZFHD) proteins. Both proteins recognize two different *cis*-acting elements in the promoter region of EARLY RESPONSE TO DEHYDRATION 1 (ERD1) (Tran et al. 2004). *Arabidopsis* ANAC072 (RD26) is induced by both dehydration and ABA, and is involved in a novel ABA-dependent stress-signaling pathway. In grasses, the NAC family is also involved in abiotic stress responses. Several rice NAC genes are induced by ABA and abiotic stresses, and the overexpression of certain NAC genes enhances drought resistance (Hu et al. 2006; Nakashima et al. 2007). MYB and MYC TFs participate in ABA-dependent pathways to upregulate abiotic stress-responsive genes. Overexpression of MYC2 and MYB2 in *Arabidopsis* induced ABA-responsive stress genes and increased osmotic stress tolerance (Abe et al. 2003).

The Antarctic hairgrass (*Deschampsia antarctica* Desv.) is one of two angiosperms endemic to the maritime Antarctica (Xiong et al. 1999; Alberdi et al. 2002). *D. antarctica*, which belongs to the subfamily Pooideae of the grass family (Poaceae), has a large and complex genome ( $2n = 26$ , 4C DNA amount =  $19.9 \pm 0.17$  pg), similar to other species of the Pooideae (Bennett et al. 1982; Cardone et al. 2009). Because this species has successfully adapted to extremely harsh environments with a wide ecological range, it has been studied in a wide range of biological fields and is a valuable resource for gene discovery associated with stress tolerance. We recently reported the first *de novo* assembly of its transcriptome using massive parallel sequencing and its expression profile using *D. antarctica* grown under various abiotic stress conditions consisting of 60,765 unigenes. Based on this study, we identified 2,353 differentially expressed genes under cold, dehydration, or salt stress (Lee et al. 2013).

In this study, using previously constructed EST database, we analyzed the TF families by examining transcripts that encode TFs and identified putative TFs that respond to abiotic stress in *D. antarctica*. We identified 1,083 TF transcripts using sequence homology to known TF gene families in seven plant species, including six monocotyledons and *Arabidopsis*, and categorized them into 53 TF families. In addition, by comparing EST abundance under stress and normal conditions, we identified a group of putative stress-induced TFs in *D. antarctica*. Overall, we provide the first comprehensive TF profile of *D. antarctica*, an important extremophile plant, providing a foundation for further systematic characterization at the single-gene or family level for genetic applications.

## Materials and methods

### Identification of TFs

TFs from *Deschampsia antarctica* were identified and organized based on sequence homology with TF sequences from seven species (*Triticum aestivum*, *Oryza sativa*, *Hordeum vulgare*, *Zea mays*, *Sorghum bicolor*, *Brachypodium distachyon*, and *Arabidopsis thaliana*) obtained from PlantTFDB 2.0 (<http://plantfdb.cbi.edu.cn/>) (Zhang et al. 2011). We subjected 60,765 putative unigene sequences of *D. antarctica* (Lee et al. 2013) to a BLASTX homology search (cutoff: transcript length  $\geq 150$  bp, BLAST score  $\geq 200$ , *E* value  $< 10^{-10}$ , and identity  $\geq 60$  %) with seven species TFs and collected the information of the top BLAST hits. To compare TF profiles from other plant species, the number of TFs that belonged to each TF family were counted and compared with the data from the PlantTFDB Website. The significance of the TF families with the expansion/contraction was statistically calculated using Fisher's exact test between the numbers of transcripts in each TF family from *D. antarctica* vs. the sum of the corresponding numbers from the other species. Gene ontology (GO) terms were assigned to each unique gene based on the sequence similarity from the BLASTX search against the nr database using the Blast2go platform. GO mapping and annotation were performed with an annotation cutoff of  $E < 1 \times 10^{-10}$ .

### Identification of stress-induced TFs

To identify stress-induced TFs, we compared the TF lists and the "DEG (differentially expressed genes) lists" from a previous study (Lee et al. 2013) and selected the TFs that were included in the DEG lists. In the previous study, to identify DEGs induced by abiotic stress in *D. antarctica*, the number of sequencing reads that supported the

consensus contigs assembled from control, cold, dehydration, and high salinity libraries was counted and divided into four categories depending on the library of origin. The permutation *t* test with a multiple testing correction indicated that 2,093 transcripts were upregulated and 1,013 transcripts were downregulated under at least one abiotic stress condition compared to the control sample, with a *q* value <0.01.

#### Quantitative real-time reverse transcription PCR (qPCR)

To prepare RNA for expression analysis, *D. antarctica* plants were vegetatively propagated in 0.5× Murashige and Skoog (MS) medium containing 2 % (w/v) sucrose under a 16:8 h light:dark cycle with a light intensity of 150 μmol m<sup>-2</sup> s<sup>-1</sup> at 16 °C (control condition) at the optimal temperature for *D. antarctica* (Salvucci and Crafts-Brandner 2004). Plants grown in either liquid or agar medium of 0.5× MS containing 2 % (w/v) sucrose were subjected to stress conditions. For dehydration and high-salinity stress treatment, the plants were grown in liquid 0.5× MS medium containing 2 % (w/v) sucrose for a week prior to the initiation of stress conditions, after which they were transferred to media containing polyethylene glycol (30 % PEG, w/v) for dehydration stress or NaCl (300 mM) for high-salinity stress, and cultured for 24 h with continuous shaking. Total RNA was extracted from leaves and purified using the RNeasy Plant Mini Kit (Qiagen, Valencia, CA, USA) according to the manufacturer's instructions. cDNA was synthesized from 1 μg of RNA extracted from samples using Superscript II (Invitrogen, Carlsbad, CA, USA). Gene-specific primers designed according to

the sequences of the contigs are listed in Table 1. QPCR was performed for triplicate samples using SYBR<sup>®</sup> Premix Ex Taq<sup>™</sup> DNA polymerase (TaKaRa, Seoul, Korea) and the Mx3000P Real-Time PCR system (Stratagene, La Jolla, CA, USA). Graph was drawn using representative results from three biological replicates.

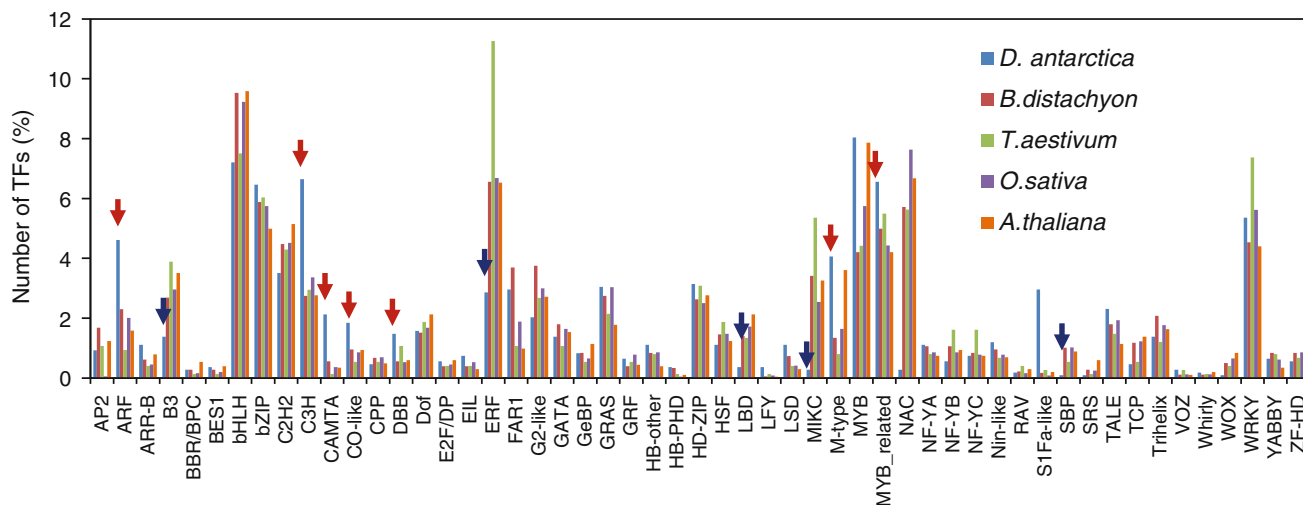
## Results

### Identification of transcripts coding TFs in *D. antarctica* ESTs

*D. antarctica* TFs were identified and organized based on sequence homology using the sequences and categories of TFs from seven species (*T. aestivum*, *O. sativa*, *H. vulgare*, *Z. mays*, *S. bicolor*, *B. distachyon*, and *A. thaliana*) from PlantTFDB 2.0, a comprehensive database of plant TFs (Zhang et al. 2011). We identified a total of 1,083 transcripts encoding TFs belonging to all 53 families (BLASTX cutoffs: transcript length ≥150 bp, score ≥200, *E* value <10<sup>-10</sup>, and identity ≥60 %) (Fig. 1). While the bHLH family was the most abundant TF group for model plants with small genomes (*A. thaliana* 9.6 %, *O. sativa* 9.2 %, *B. distachyon* 9.5 %), the MYB-related family (87, 8.0 %) was the most abundant in *D. antarctica*, followed by the bHLH (78, 7.2 %), C3H (72, 6.6 %), NAC (71, 6.6 %), and bZIP (70, 6.5 %; Table 2). Our distribution analysis indicated that the overall distribution of transcripts encoding the various known TF families was very similar with that of other model species (Fig. 1). However, specific families showed expansion (e.g., ARF, C3H, CAMTA, CO-like, DBB, M-type, and MYB-related) or contraction

**Table 1** The unigene IDs and primer sequences for genes used in this study

Unigene ID	Sequences (5′–3′)		Amplicon size
	Forward	Reverse	
CL871Contig2	ACATCACATCACACTGCAAT	AAGCTGACTAGCCATCCTAG	123
CL1192Contig1	CTGTCCTCGACGCAGAAGAT	TGCGACGTCGAGATCCAC	137
CL1316Contig1	TTTACAGAGCTAGTGTGGCG	AGGACTTGATCGGCAGAGAC	120
CL1670Contig1	TTGGAGCTCACTGCAGCAGC	CCATGGCCTGCCTGACTTTG	150
CL1977Contig1	CAGCTAAAGACCAATCAGTCAACC	ACCGCCAGCTGTCTGATT	134
CL2008Contig1	CTAGTTATAGCATGTCAAGC	GAATCCACAAGGACCTTCT	135
CL2221Contig1	GCGCCGTCGCTGTGCAAAC	CGACCACCTTCTCCACGCCG	150
CL2357Contig2	GTCGCGCAGACCATCCAGAT	ACGGTGGGGCCCTCCTGAC	170
CL2509Contig1	TGCACCATCTCTAGCTGACT	ACCACCCGGCTTCCTTTAGG	122
CL2522Contig1	GGGCATTTCCAGATTCCGT	GAAGCGTCCACGGAATCG	125
CL2573Contig1	ACCATCCAACAGGAACCTTCATG	TGCTTGCCATCCATTCCGA	149
CL3052Contig1	CCGTTTCAGATCCAGATCA	ATCGCACGCTGAAGATGG	146
CL3326Contig1	ACTGTGACCGAGAAAACGCT	CATATGGGCAACTCTTGGC	146
CL3554Contig1	TCAAATTTGTCCCGTCC	TTCCCAATGTTGGCAAGG	150



**Fig. 1** Distribution of TF-encoding genes/transcripts of *D. antarctica* and other plant species in different TF families. The numbers of TF genes from *B. distachyon*, *T. aestivum*, *O. sativa*, and *A. thaliana* were obtained from PlantTFDB 2.0 (<http://planttfdb.cbi.edu.cn/>) (Zhang

et al. 2011). TF families that showed significantly different frequencies between *D. antarctica* and other species are shown in red or dark blue color arrows (red expansion, dark blue contraction,  $p$  value  $<0.01$  using Fisher's exact test)

**Table 2** The number of transcripts in each TF family identified in *D. antarctica*

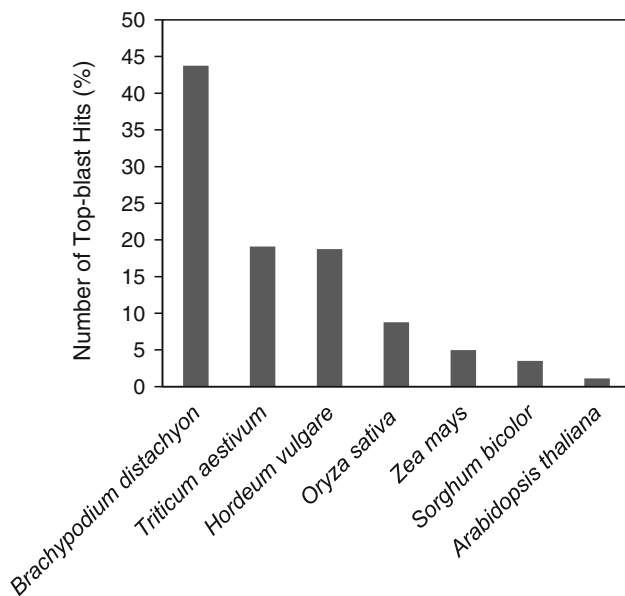
TF family	No. unigenes	(%)	TF family	No. unigenes	(%)	TF family	No. unigenes	(%)
MYB_related	87	8.0	bHLH	78	7.2	C3H	72	6.6
NAC	71	6.6	bZIP	70	6.5	WRKY	58	5.4
ARF	50	4.6	MYB	44	4.1	C2H2	38	3.5
HD-ZIP	34	3.1	GRAS	33	3.0	FAR1	32	3.0
SBP	32	3.0	ERF	31	2.9	TALE	25	2.3
CAMTA	23	2.1	G2-like	22	2.0	CO-like	20	1.8
Dof	17	1.6	DBB	16	1.5	B3	15	1.4
GATA	15	1.4	Trihelix	15	1.4	Nin-like	13	1.2
ARR-B	12	1.1	HB-other	12	1.1	HSF	12	1.1
MIKC	12	1.1	NF-YA	12	1.1	AP2	10	0.9
GeBP	9	0.8	EIL	8	0.7	NF-YC	8	0.7
GRF	7	0.6	YABBY	7	0.6	E2F/DP	6	0.6
NF-YB	6	0.6	ZF-HD	6	0.6	CPP	5	0.5
TCP	5	0.5	BES1	4	0.4	HB-PHD	4	0.4
LBD	4	0.4	LSD	4	0.4	BBR/BPC	3	0.3
M-type	3	0.3	NF-X1	3	0.3	VOZ	3	0.3
RAV	2	0.2	Whirly	2	0.2	SRS	1	0.1
STAT	1	0.1	WOX	1	0.1			

(e.g., B3, ERF, LBD, MIKC, and SBP) events (Fisher's exact  $p$  value  $<0.01$ ), suggesting that differences in the abundance of TF families might play a role in regulating species-specific biological processes with evolutionary significance in *D. antarctica*. The top-hit species distribution of BLASTX matches to TF protein sequences is shown in Fig. 2. A significant proportion of *D. antarctica* ESTs showed best-matches with TF proteins of *B. distachyon* (474/1,083, 43.8 %), followed by *T. aestivum* (203/1083,

18.7 %), suggesting that overall sequences of TF genes of *D. antarctica* may be more similar to *B. distachyon* than to *T. aestivum*.

#### GO distribution of identified TFs of *D. antarctica*

To investigate putative regulatory functions of the identified TFs, we mapped GO terms for the 1,083 transcripts based on sequence similarity from the BLASTX search



**Fig. 2** Species distribution represented by the top BLAST hits of *D. antarctica* TF homologs

against the nr database using the Blast2go platform. Among 923 GO-annotated TFs, several analyzed TFs were related to various biological processes such as abiotic stimulus response, defense response, hormone response, and organ development, indicating that they may control various biological processes (Table 3). Considering the response to environmental stimuli, cold stress signaling proteins such as CAMTA (CL10091Contig1, CL13908Contig1) and ICE1 (CL20383Contig1), and disease resistance proteins such as ERF (CL699Contig1, CL5844Contig1), TGA/OBF proteins (CL5402Contig2, CL8974Contig1) and WRKY33 (CL16951Contig1) were included in the GO categories of “response to abiotic stress” and “response to biotic stress”, respectively. A number of TFs encoding light signaling proteins and hormone signaling proteins were also included in our GO functional categorization. For example, TFs such as HY5 (CL2857Contig2), FAR1-related (CL22976Contig1), and HD1 (CL12566Contig1) were included in the “response to light stimuli”. TFs such as ABI5 (CL1754Contig2) and OCP3 (CL1477Contig1), several auxin response factors (e.g., CL19544Contig1, CL795Contig1, CL2018Contig2, CL5007Contig1, CL10534Contig1), and GAI (CL2576Contig1), were included in the GO categories of “response to ABA,” “response to auxin,” and “response to gibberellins,” respectively. In addition, many TF proteins related to “developmental processes” were also identified. Floral homeotic proteins AP2 (CL3100Contig1) and Ta-VRT2-like (CL19603Contig1) were included in the GO category of “flower development.” TFs such as DL-like (DLOOPING LEAF) protein (CL18256Contig1), YABBY-like protein (CL4511Contig1), and GL2-like

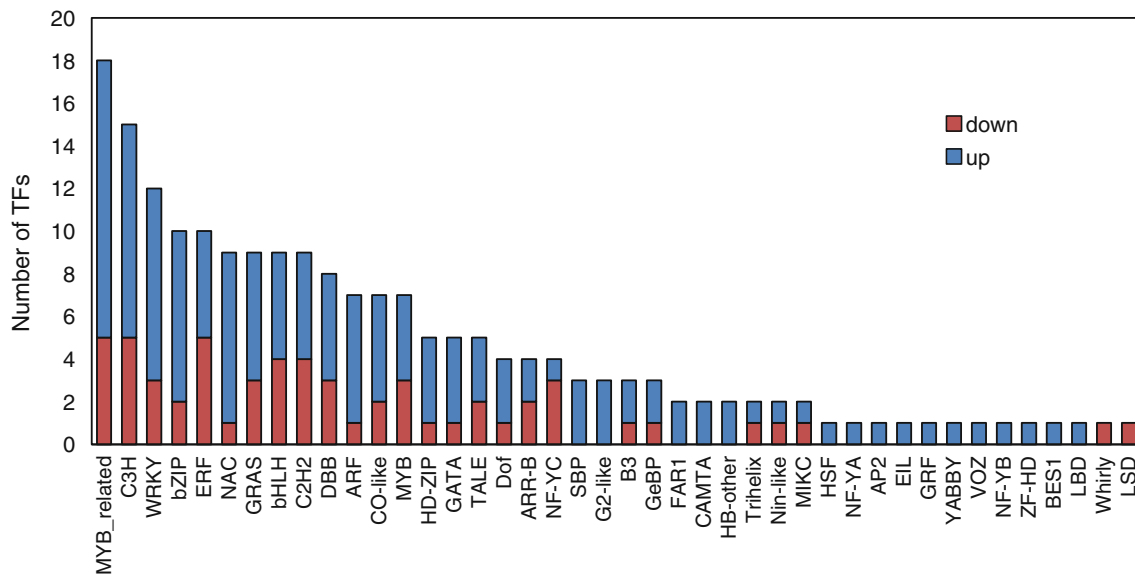
**Table 3** Distribution of TFs for the representative GO terms

GO terms	GO ID	No. unigenes
Terms for response to stimulus		
Response to hormone stimulus	GO:0009725	70
Response to stress	GO:0006950	57
Response to abiotic stimulus	GO:0009628	54
Response to auxin stimulus	GO:0009733	41
Response to light stimulus	GO:0009416	30
Response to radiation	GO:0009314	30
Response to biotic stimulus	GO:0009607	24
Response to other organism	GO:0051707	23
Response to bacterium	GO:0009617	16
Response to jasmonic acid stimulus	GO:0009753	14
Response to salt stress	GO:0009651	14
Response to osmotic stress	GO:0006970	14
Response to inorganic substance	GO:0010035	13
Response to abscisic acid stimulus	GO:0009737	13
Response to salicylic acid stimulus	GO:0009751	11
Response to gibberellin stimulus	GO:0009739	10
Response to carbohydrate stimulus	GO:0009743	10
Response to red or far red light	GO:0009639	10
Response to metal ion	GO:0010038	9
Response to temperature stimulus	GO:0009266	9
Response to ethylene stimulus	GO:0009723	9
Response to water deprivation	GO:0009414	8
Response to DNA damage stimulus	GO:0006974	7
Immune response	GO:0006955	7
Response to cadmium ion	GO:0046686	6
Response to UV	GO:0009411	5
Response to light intensity	GO:0009642	5
Terms for developmental processes		
Reproduction	GO:0000003	32
Organ development	GO:0048513	24
Flower development	GO:0009908	15
Shoot development	GO:0048367	9
Embryonic development	GO:0009790	7
Fruit development	GO:0010154	7
Leaf development	GO:0048366	5
Seed development	GO:0048316	6
Senescence	GO:0010149	8

protein (FGW8HAD01DBHP0), known to play roles in leaf development, were included in the GO category of “leaf development.”

#### Expression analysis of the stress-induced TFs

EST abundance based on next-generation sequencing results has been widely used for the identification and quantification of mRNA species under different conditions or in different cell types. For ESTs that mapped to the TFs,



**Fig. 3** Distribution of stress-responsive TF-encoding transcripts of *D. antarctica* in different TF families

we compared the expression index of the each transcript under control versus abiotic stress conditions. We found that 136 TFs were upregulated and 58 TFs were down-regulated by low temperature, dehydration, or high salinity ( $q$  value  $<0.05$ ; Fig. 3). Stress-induced TF proteins included the well-known stress-responsive genes such as DREB homologs (CL10747Contig1, CL6273Contig1), ERF4 homolog (CL3052Contig1), ABI5 (a member of bZIP TF) homologs (CL2221Contig1, CL2357Contig2), and NAC1/NAC2 homologs (CL835Contig1/CL193Contig1). Other known genes in this group are LHY (CL64Contigs), ARFs (CL2018Contig2, CL7485Contig, CL795Contig1, CL5007Contig1, CL5536Contig1, CL2133Contig1), and BZR1 (CL3575Contig1), regulated by circadian clock, auxin, and brassinosteroid, respectively. In addition, many genes encoding putative zinc finger proteins (10 C3H, 5 C2H2, 5 DBB, 3 DOF), MYB-related, WRKY, bZIPs, AP2/ERF, NAC, and GRAS TF families were also identified. While the majority of TFs were upregulated by specific stress (cold: 40, dehydration: 37, salt: 42), some were commonly induced in response to cold–salt, cold–dehydration, dehydration–salt, or cold–dehydration–salt combinations. Among those, the transcripts level of ERF4 (CL3052Contig1), a NAC domain protein (CL871Contig2), and a putative WRKY family gene (CL2522 Contig1), were upregulated by all three stresses (Table 4).

#### Validation of stress-induced TFs by qPCR analysis

To validate the induction of transcripts by abiotic stresses, more than five genes per each specific abiotic stress were chosen and tested by qPCR analysis. Figure 4 shows the gene expression levels of TF-encoding transcripts in

*D. antarctica* when exposed to cold, drought, or high salinity for 24 h as compared to the control samples with no treatment. Of the transcripts, CL1670Contig1 encoding a NAC showed a strong increase by both salt and dehydration ( $>8$ -fold), and the CL3052Contig1 encoding a ERF4 was increased by all stresses, as predicted. Since many of the TFs involved in stress acclimation showed an acute response upon stress treatment (Kreps et al. 2002), examination of the expression kinetics with different durations of stress treatments, different stress levels, or different developmental stages is required.

#### Discussion

Wild plant species, which have evolved a high level of tolerance toward environmental stresses, represent important genetic resources that can be used to improve crop resistance against global warming. *D. antarctica* is an important flora extremophile that survives on marginal land and has been studied extensively for several decades (Lewis Smith 1994). Although physiological characteristics have improved our understanding of the *D. antarctica* stress-tolerance mechanism, little gene-based information is available. At this time, 38 cDNAs have been cloned and three genes (*DaGrx*, *DaRub1*, and *DaPyk1*) were characterized using a combination of proteomics and mRNA differential display from *D. antarctica* (Gidekel et al. 2003). A total of 1,199 unigene clusters were identified from two cDNA libraries originated from greenhouses and Antarctica exhibiting different expression patterns of several stress-responsive genes depending on environmental conditions (Lee et al. 2008). A Cu/Zn SOD gene was

**Table 4** The list of stress-induced TFs in *D. antarctica*

TF family	EST ID	Length (bp)	Description	Best Match <sup>a</sup>	E value	Stress <sup>b</sup>
AP2	CL7719Contig1	330	AP2D23-like protein	Bdi015502	$2.7 \times 10^{-22}$	C
ARF	CL2133Contig1	790	Auxin response factor	Bdi028017	$1.1 \times 10^{-79}$	S
ARF	CL5007Contig1	677	Auxin response factor	Bdi017582	$1.6 \times 10^{-60}$	C
ARF	CL795Contig1	1,377	Auxin response factor	Bdi017757	$6.4 \times 10^{-146}$	C
ARF	CL2018Contig2	455	Auxin response factor 1	Bdi000039	$8.7 \times 10^{-60}$	C, S
ARF	CL7485Contig1	378	Auxin response factor 1	Bdi026948	$4.0 \times 10^{-69}$	C
ARF	CL5536Contig1	480	Auxin response factor	Bdi008680	$7.4 \times 10^{-36}$	S
ARR-B	CL6160Contig1	360	Response regulator 10	Hvu002699	$1.7 \times 10^{-50}$	C
ARR-B	CL7777Contig1	668	Response regulator 10	Bdi028669	$1.7 \times 10^{-54}$	C
B3	CL1951Contig1	514	Protein	Bdi010263	$1.0 \times 10^{-73}$	D, S
B3	CL760Contig1	733	Protein	Tae012357	$1.1 \times 10^{-59}$	C
BES1	CL3575Contig1	715	BZR1 protein	Bdi009462	$4.8 \times 10^{-87}$	C, S
bHLH	CL10445Contig1	234	bHLH protein	Bdi004186	$1.2 \times 10^{-33}$	S
bHLH	CL13922Contig1	245	bHLH protein	Bdi026065	$1.0 \times 10^{-29}$	D
bHLH	CL3741Contig1	456	bHLH protein	Bdi002420	$1.4 \times 10^{-44}$	S
bHLH	CL4172Contig1	517	bHLH protein like	Hvu002587	$4.1 \times 10^{-22}$	D
bHLH	CL9017Contig1	593	F-box family protein	Osj050834	$9.0 \times 10^{-30}$	C
bZIP	CL2221Contig1	574	ABI5	Tae000681	$3.1 \times 10^{-48}$	D, S
bZIP	CL2357Contig2	599	ABI5 like	Bdi017510	$3.6 \times 10^{-55}$	S
bZIP	CL2058Contig1	660	bZIP protein	Hvu001017	$4.3 \times 10^{-39}$	C
bZIP	CL11271Contig1	419	bZIP transcription factor	Bdi019980	$1.0 \times 10^{-37}$	C
bZIP	CL4951Contig1	312	bZIP transcription factor	Tae003937	$5.0 \times 10^{-45}$	D
bZIP	CL5907Contig1	385	bZIP transcription factor	Hvu003231	$2.9 \times 10^{-17}$	S
bZIP	CL8974Contig1	475	bZIP transcription factor	Bdi003244	$7.5 \times 10^{-25}$	C
bZIP	CL9592Contig1	215	bZIP transcription factor	Tae002104	$3.2 \times 10^{-36}$	C
C2H2	CL10316Contig1	227	C2H2 zinc finger protein	Osj025467	$6.1 \times 10^{-22}$	D
C2H2	CL6126Contig1	245	C2H2 zinc finger protein	Hvu006316	$1.2 \times 10^{-22}$	S
C2H2	CL3239Contig1	719	Protein	Sbi031465	$9.7 \times 10^{-72}$	S
C2H2	CL14848Contig1	299	Stress-associated 11	Hvu004683	$1.8 \times 10^{-26}$	C
C2H2	CL4646Contig1	547	TRM repressor protein	Hvu003727	$2.1 \times 10^{-43}$	S
C3H	CL6125Contig1	220	C3H domain protein 16	Bdi000314	$5.5 \times 10^{-20}$	S
C3H	CL11680Contig1	501	C3H domain protein 64	Hvu003800	$9.6 \times 10^{-29}$	C
C3H	CL4048Contig1	478	C3H type Znf protein	Osj025086	$2.5 \times 10^{-60}$	C
C3H	CL11535Contig1	233	c-x8-c-x5-c-x3-h type family protein	Osj038202	$3.8 \times 10^{-24}$	C
C3H	CL15321Contig1	237	c-x8-c-x5-c-x3-h type family protein	Bdi007705	$3.4 \times 10^{-17}$	D
C3H	CL10012Contig1	233	Protein	Bdi020319	$1.2 \times 10^{-38}$	S
C3H	CL1977Contig1	717	Zfn-like 1	Tae000858	$3.1 \times 10^{-17}$	C, D
C3H	CL7212Contig1	518	Zinc finger	Osj015920	$4.3 \times 10^{-35}$	S
C3H	CL10671Contig1	405	Zinc finger family protein	Hvu004030	$1.3 \times 10^{-47}$	D
C3H	CL1761Contig1	1,177	Zinc finger protein	Hvu010937	$4.9 \times 10^{-176}$	S
CAMTA	CL1316Contig1	742	CAMTA family protein	Bdi005706	$3.4 \times 10^{-67}$	S
CAMTA	CL4241Contig1	609	CAMTA family protein	Sbi025191	$8.3 \times 10^{-47}$	D
CO-like	CL132Contig10	486	Constans-like protein CO6	Osj050357	$6.7 \times 10^{-32}$	D
CO-like	CL12566Contig1	228	Heading date1	Osj051756	$1.1 \times 10^{-18}$	D
CO-like	CL743Contig3	871	Protein	Osj046526	$1.2 \times 10^{-48}$	C
CO-like	CL132Contig11	1,299	Zinc finger protein	Hvu003969	$5.3 \times 10^{-102}$	S
CO-like	CL1703Contig2	387	Zinc finger protein	Osj008444	$4.3 \times 10^{-29}$	S
DBB	CL575Contig1	674	Protein	Bdi029851	$5.8 \times 10^{-55}$	S

**Table 4** continued

TF family	EST ID	Length (bp)	Description	Best Match <sup>a</sup>	<i>E</i> value	Stress <sup>b</sup>
DBB	CL575Contig3	644	Protein	Tae009529	$2.9 \times 10^{-77}$	C, S
DBB	CL117Contig1	761	Zinc finger protein	Hvu004367	$2.8 \times 10^{-56}$	S
DBB	CL1192Contig1	363	Zinc finger protein	Hvu017563	$8.2 \times 10^{-45}$	C, S
DBB	CL4356Contig1	733	Zinc finger protein	Bdi019086	$6.3 \times 10^{-58}$	D
Dof	CL3326Contig1	730	Cycling dof factor-like 1	Bdi007116	$4.6 \times 10^{-45}$	C
Dof	CL9282Contig1	452	Dof zinc finger protein	Hvu007246	$2.5 \times 10^{-43}$	D
Dof	CL2008Contig1	304	Zinc finger protein	Sbi031102	$7.3 \times 10^{-36}$	C
EIL	CL633Contig1	953	EIN3-like 1 expressed	Hvu001687	$4.0 \times 10^{-77}$	C
ERF	CL10747Contig1	549	DREB	Bdi020982	$1.4 \times 10^{-39}$	D
ERF	CL6273Contig1	565	DREB2	Tae000338	$2.5 \times 10^{-71}$	D
ERF	CL2573Contig1	807	ERE binding protein	Bdi024722	$6.5 \times 10^{-75}$	D
ERF	CL3052Contig1	751	ERF4	Bdi029072	$5.0 \times 10^{-26}$	C, D, S
ERF	CL90Contig7	764	Root abundant factor	Hvu000656	$6.8 \times 10^{-42}$	C
FAR1	CL14727Contig1	233	FAR protein like	Bdi023493	$1.7 \times 10^{-40}$	D
FAR1	CL15098Contig1	425	FAR protein like	Bdi017686	$5.1 \times 10^{-24}$	C
G2-like	CL2557Contig1	800	MYB cc transcription factor	Hvu010644	$2.6 \times 10^{-31}$	S
G2-like	CL11932Contig1	240	MYB transcription factor	Bdi012196	$4.0 \times 10^{-18}$	D
G2-like	CL3860Contig2	719	MYB transcription factor	Hvu005236	$6.5 \times 10^{-60}$	S
GATA	CL10444Contig1	460	GATA transcription factor	Bdi016675	$4.6 \times 10^{-48}$	C
GATA	CL7163Contig1	236	GATA transcription factor	Osj027368	$1.1 \times 10^{-18}$	D
GATA	CL1849Contig2	385	GATA4	Bdi018495	$1.2 \times 10^{-44}$	C
GATA	CL4645Contig2	335	Protein	Tae011011	$2.0 \times 10^{-41}$	S
GeBP	CL9171Contig1	405	Protein	Ath010279	$3.0 \times 10^{-31}$	D
GeBP	CL9748Contig1	255	Storekeeper protein	Bdi024725	$2.5 \times 10^{-20}$	C
GRAS	CL2576Contig1	497	GAI	Tae002005	$3.9 \times 10^{-75}$	C
GRAS	CL1967Contig1	765	GRAS transcription factor	Bdi007245	$1.3 \times 10^{-90}$	S
GRAS	CL3160Contig1	782	GRAS transcription factor	Hvu001960	$1.8 \times 10^{-93}$	S
GRAS	CL6782Contig1	358	GRAS transcription factor	Bdi007245	$1.3 \times 10^{-60}$	C
GRAS	CL7344Contig1	554	GRAS transcription factor	Osj049006	$4.8 \times 10^{-51}$	D
GRAS	CL3762Contig1	341	SCL1 protein	Bdi019888	$3.2 \times 10^{-29}$	S
GRF	CL7647Contig1	330	Growth-regulating factor 8	Bdi028800	$4.0 \times 10^{-50}$	S
HB-other	CL2509Contig1	247	Homeobox-like resistance	Tae006784	$3.5 \times 10^{-30}$	S
HB-other	CL11111Contig1	349	Homeodomain-like transcriptional regulator	Bdi017079	$8.8 \times 10^{-51}$	D
HD-ZIP	CL4126Contig2	634	DNA-binding protein	Hvu005452	$5.4 \times 10^{-60}$	S
HD-ZIP	CL2201Contig2	570	HD-zip protein	Bdi020278	$3.9 \times 10^{-19}$	S
HD-ZIP	CL365Contig3	1,274	HD-zip protein cphb-5	Bdi016785	$7.2 \times 10^{-96}$	C
HD-ZIP	CL9983Contig1	243	HD-zip protein roc6	Bdi015114	$3.4 \times 10^{-41}$	S
HSF	CL7019Contig1	377	Heat shock factor	Bdi006519	$1.3 \times 10^{-48}$	D
LBD	CL7060Contig1	302	Asymmetric leaves2	Tae007185	$5.8 \times 10^{-25}$	C
MIKC	CL3025Contig1	694	Short vegetative phase	Tae009496	$5.6 \times 10^{-53}$	C
MYB	CL12805Contig1	228	Hypothetical protein	Bdi011672	$1.7 \times 10^{-19}$	D
MYB	CL11586Contig1	215	MYB transcription factor	Osj024318	$1.5 \times 10^{-33}$	C
MYB	CL2993Contig2	521	MYB-related protein	Hvu004678	$2.0 \times 10^{-24}$	D, S
MYB	CL5205Contig2	397	R2R3 MYB protein	Tae005147	$3.6 \times 10^{-34}$	D
MYB_related	CL64Contig4	1,378	Circadian oscillator component	Hvu001561	$5.0 \times 10^{-143}$	D, S
MYB_related	CL64Contig2	1,336	COC1	Hvu001561	$1.9 \times 10^{-118}$	S
MYB_related	CL2456Contig1	331	Initiator-binding expressed	Ath028434	$1.2 \times 10^{-17}$	S
MYB_related	CL64Contig1	459	LHY protein	Tae001592	$2.5 \times 10^{-62}$	S



**Table 4** continued

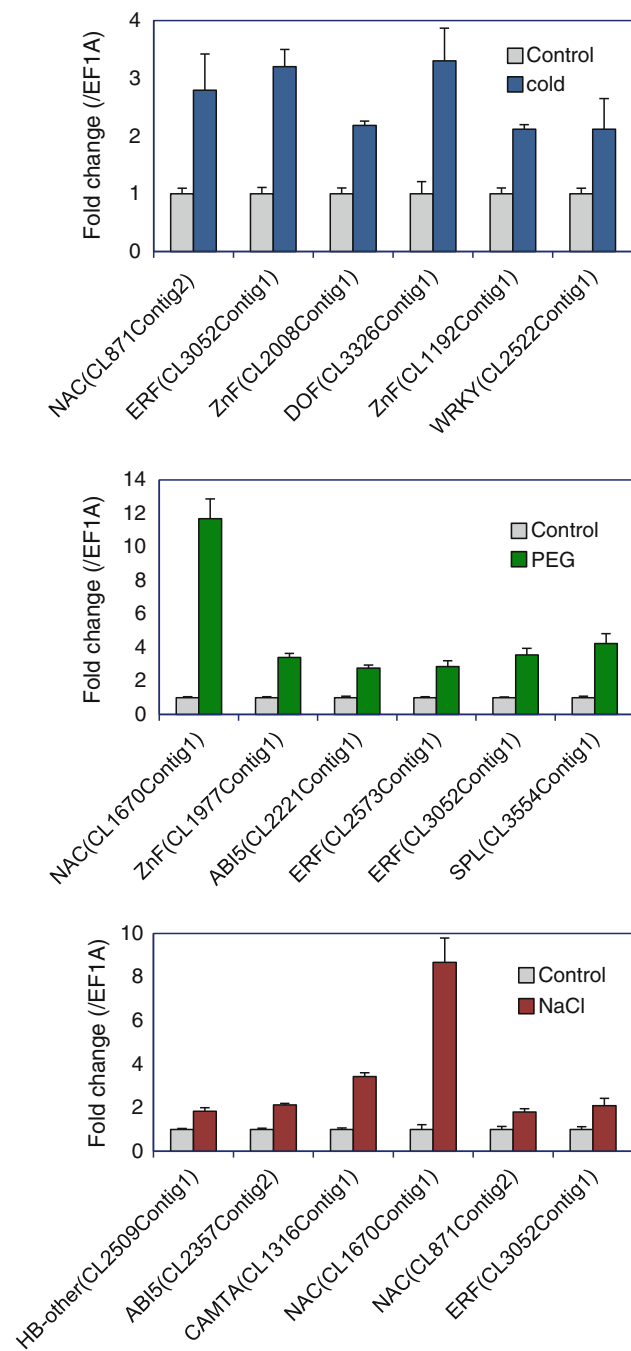
TF family	EST ID	Length (bp)	Description	Best Match <sup>a</sup>	E value	Stress <sup>b</sup>
MYB_related	CL64Contig3	712	LHY protein	Hvu001561	$4.6 \times 10^{-58}$	D, S
MYB_related	CL1769Contig1	660	MYB transcription expressed	Bdi003750	$5.4 \times 10^{-42}$	C
MYB_related	CL1386Contig3	491	MYB transcription factor	Bdi017728	$8.0 \times 10^{-65}$	S
MYB_related	CL38Contig7	745	MYB transcription factor	Osj026179	$5.3 \times 10^{-20}$	S
MYB_related	CL6896Contig1	597	MYB transcription factor 3	Tae005264	$1.4 \times 10^{-67}$	C
MYB_related	CL6716Contig1	233	MYB29 protein	Bdi025026	$7.4 \times 10^{-20}$	S
MYB_related	CL14777Contig1	250	MYB-like	Tae000299	$2.1 \times 10^{-27}$	C
MYB_related	CL1604Contig2	842	R2R3 MYB protein	Tae003202	$1.9 \times 10^{-104}$	D, S
MYB_related	CL2456Contig4	376	Telomere repeat-binding protein 5	Ath028434	$4.4 \times 10^{-36}$	D
NAC	CL1670Contig1	812	NAC domain ipr003441	Bdi023098	$7.0 \times 10^{-85}$	D, S
NAC	CL7154Contig1	436	NAC domain ipr003441	Bdi029479	$1.4 \times 10^{-35}$	S
NAC	CL835Contig1	1,068	NAC1 transcription factor	Hvu003954	$1.4 \times 10^{-94}$	D
NAC	CL193Contig1	1,301	NAC2 protein	Tae001896	$1.0 \times 10^{-73}$	C, S
NAC	CL14981Contig1	243	NAC5 protein	Hvu017189	$2.1 \times 10^{-43}$	C
NAC	CL871Contig2	797	NAC68	Tae007889	$1.9 \times 10^{-63}$	C, D, S
NAC	CL8752Contig1	532	NAM (no apical meristem) protein	Bdi010078	$8.2 \times 10^{-21}$	D
NAC	CL3291Contig2	725	Protein	Bdi012610	$6.3 \times 10^{-18}$	S
NF-YA	CL4574Contig1	701	Nuclear transcription factor y subunit a-10	Bdi027867	$3.1 \times 10^{-38}$	C
NF-YB	CL9000Contig1	243	Nuclear transcription factor y subunit b-3	Bdi020963	$4.0 \times 10^{-26}$	C
NF-YC	CL8605Contig1	282	Nuclear transcription factor y subunit c-2	Sbi016872	$9.1 \times 10^{-47}$	D
Nin-like	CL5240Contig1	223	RWP-RK domain	Hvu001374	$1.4 \times 10^{-26}$	D
SBP	CL3554Contig1	255	SPL protein	Hvu001410	$1.5 \times 10^{-38}$	D
SBP	CL6058Contig2	244	SPL9	Bdi004027	$2.8 \times 10^{-27}$	S
SBP	CL7311Contig1	496	Squamosa promoter binding 12	Bdi003965	$2.1 \times 10^{-57}$	C
TALE	CL10435Contig1	237	HD protein	Tae002173	$9.0 \times 10^{-18}$	S
TALE	CL11068Contig1	216	KN1 homeobox protein	Bdi013846	$2.2 \times 10^{-40}$	S
TALE	CL263Contig5	1,193	KNOX family class 2	Hvu006567	$1.7 \times 10^{-112}$	C, S
Trihelix	CL9054Contig1	470	6b-interacting protein 1	Bdi024147	$1.1 \times 10^{-20}$	D
VOZ	CL4459Contig1	553	Vascular plant one zinc finger protein	Bdi026708	$7.3 \times 10^{-44}$	D
WRKY	CL4495Contig1	860	SUSIBA2-like protein	Hvu002074	$9.9 \times 10^{-133}$	D
WRKY	CL14825Contig1	359	WRKY transcription factor	Tae009827	$1.1 \times 10^{-46}$	D
WRKY	CL2522Contig1	706	WRKY transcription factor	Tae002244	$2.4 \times 10^{-43}$	C, D, S
WRKY	CL5364Contig1	600	WRKY transcription factor	Bdi005086	$6.0 \times 10^{-42}$	D
WRKY	CL5366Contig1	412	WRKY transcription factor	Bdi024049	$6.7 \times 10^{-50}$	S
WRKY	CL6467Contig1	343	WRKY transcription factor	Bdi015774	$4.3 \times 10^{-34}$	D
WRKY	CL11486Contig1	384	WRKY7	Hvu000275	$9.7 \times 10^{-50}$	D
WRKY	CL1847Contig3	761	WRKY74	Hvu003707	$1.4 \times 10^{-42}$	D
WRKY	CL4181Contig1	457	WRKY78	Tae002039	$2.9 \times 10^{-47}$	S
YABBY	CL4511Contig1	489	YABBY protein	Bdi009091	$1.8 \times 10^{-48}$	C
ZF-HD	CL14100Contig1	298	ZF-HD homeobox protein	Osj003099	$6.5 \times 10^{-32}$	C

<sup>a</sup> The ID of TF collected in PlantTFDB. The TF ID consists of three characters which represent the species (e.g. Bdi, *B. distachyon*; Osj, *O. sativa japonica*; Hvu, *H. vulgare*; Tae, *T. aestivum*; Sbi *S. bicolor*; Ath, *A. thaliana*)

<sup>b</sup> The abiotic stress which induces the transcripts level for the corresponding contig (C cold, D dehydration, S high salinity)

cloned to account for the ability of *D. antarctica* to tolerate high levels of oxidative stress (Sanchez-Venegas et al. 2009). Ice recrystallization inhibition protein homologs were cloned and the dynamic changes in their gene expression during cold acclimation were demonstrated

(John et al. 2009; Chew et al. 2012). Recently, we collected EST data from stress-treated mRNA libraries of *D. antarctica* using GS 454-FLX pyrosequencing (Lee et al. 2013). This dataset increased our understanding regarding the genetic applications of *D. antarctica*. In this study, 1,083



**Fig. 4** qPCR analysis of selected *D. antarctica* stress-induced TFs following exposure to different abiotic stressors. Statistical significance of gene induction by comparison with control was assessed using *t* test ( $p < 0.05$ ). Gray and color bars represent control and treatment samples, respectively. The vertical axis indicates the relative ratio of the transcript abundance of the selected gene compared to the abundance of the transcripts of the internal *DaEF1a1* (Lee et al. 2010). Data and error bars represent means and standard deviations, respectively, of three technical replicates. Total RNA was extracted from samples of plants grown under control conditions (16 °C no treatment) or exposed to a low temperature (cold 4 °C), dehydration (PEG 30 %), or high salinity (NaCl 300 mM). Three biological replicates were performed for each experiment, with similar results

transcripts encoding TFs belonging to 53 families were identified based on sequence homology between *D. antarctica* EST and the TF database of seven plant species.

Characterizing the function of stress-inducible genes is very important to elucidate the molecular mechanisms of stress response and tolerance of higher plants and to improve the stress tolerance of crops through bioengineering. Gene regulation by TFs is an important step of the stress-responsive signal transduction cascades. Thus, stress-responsive TFs play a crucial role in turning on/off gene expression required for stress acclimation. Based on in silico analysis of the EST database of *D. antarctica*, we identified 127 transcripts encoding putative TFs, which were upregulated by abiotic stresses. These genes include the homolog transcripts encoding proteins such as ARF, bHLH, bZIP, zinc finger families (C2H2, C3H, CO-like, DBB, and Dof), AP2/ERF, GATA, GRAS, HD-zip, MYB-related, NAC, and WRKY. These genes have been characterized as key factors in various stress signaling pathways.

Members of DREB from the AP2/ERF family are also stress-inducible and mediate the transcription of several genes such as *rd29A*, *rd17*, *cor15a*, *erd10*, *kin1*, *kin2*, and others in response to cold and water stress (Liu et al. 1998; Thomashow 1999). *AtERF4* is induced by ABA and high salinity, and acts as a transcriptional regulator in ABA signaling, which plays an important role in adaptive stress responses to environmental stimuli in plants (Yang et al. 2005). In the stress-induced TF list, ABI5 homologs and bZIP family TFs were included, as well as ABI3 homologs and B3 family TFs, which might play a role in ABA signaling. In *Arabidopsis*, the ABA response is regulated by TFs such as ABI5 and ABI3 (Giraudat et al. 1992; Finkelstein and Lynch 2000). Several studies have shown that ABI5 and ABI3 interact and bind to AREB elements and co-regulate downstream ABA-responsive genes (Nakamura et al. 2001; Lopez-Molina et al. 2002; Nakashima et al. 2006). In addition, we identified 11 contigs encoding NAC family members in the stress-induced TF list. ANAC019, ANAC055, and ANAC072 in *Arabidopsis* and several NAC TFs in grass species were shown to be induced by ABA, high salinity, and dehydration, and transgenic plants overexpressing these genes have increased drought tolerance (Tran et al. 2004; Hu et al. 2006; Nakashima et al. 2007; Hu et al. 2008; Zheng et al. 2009). This suggests that NAC homologs may play a similar role for stress tolerance in *D. antarctica*.

In other plants, similar expression profiles of TFs have been reported; several families of TFs, each containing a distinct type of DNA-binding domain and several classes of zinc finger domains, have been implicated in plant stress responses because their expression is induced or repressed

under different stress conditions in *Arabidopsis* (Chen et al. 2002; Jiang and Deyholos 2006). For example, MYB genes, AP2/EREBP genes, bHLH genes, HSF genes, NAC genes, and WRKY genes are affected by abiotic stresses in rice (Rabbani et al. 2003). Also, AP2/ERF, MYB, NAC, and WRKY are affected in common wheat, a Triticeae species, which possesses more tolerance for abiotic stress than rice or maize (Kawaura et al. 2008), suggestive of functional conservation of these TF families in the plant stress response. The similarities of TF expression patterns under abiotic stress in a wide range of plant lineages imply that overall molecular response to environmental change is evolutionarily conserved between model species and other extremophiles.

Many eukaryotic genomes have experienced multiple large-scale duplication events during their evolutionary history (Lespinet et al. 2002; Richardt et al. 2007). Evolutionary retention of duplicated genes encoding transcription-associated proteins may be positively correlated with increasing morphological complexity, especially within the plant kingdom (Lespinet et al. 2002; Shiu et al. 2005). Hence, the cross-species comparisons of TFs is expected to yield novel insights into the evolution of regulatory networks in plants (Lang et al. 2010). Our TF profile analysis showed that the distribution of TF families was generally similar with those of other species. However, several cases of expansion and contraction of some families were also observed in *D. antarctica*. For example, *D. antarctica* had significantly more ARF, C3H, CAMTA, and S1Fa-like TFs than other species, while it had significantly fewer ERF and MIKC TFs, suggesting that these differences in the abundance of TFs in *D. antarctica* might play an important role in regulating species-specific biological processes with evolutionary implications. To exclude any possible biased observation and to generate an accurate explanation for the expansion and contraction events with an evolutionary view, the homology-based search using EST databases should be combined with a motif-based search (e.g., prediction method using Hidden Markov Models from the PFAM database) using the complete genome sequences or full-length ORFome data with sufficient coverage of the *D. antarctica* genome.

QPCR suggested that the selected TFs control the downstream genes in stress signal transduction pathways, providing a guideline for functional characterization of these genes. To further study and identify the molecular function of TF candidates, molecular and genetic analysis, such as cloning the full sequence of ORFs and promoter sequences, investigating the dynamics of gene expression with various stress conditions, and characterizing in vivo function using transgenic plants, should be performed.

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## References

- Abe H, Urao T, Ito T, Seki M, Shinozaki K, Yamaguchi-Shinozaki K (2003) *Arabidopsis* AtMYC2 (bHLH) and AtMYB2 (MYB) function as transcriptional activators in abscisic acid signaling. *Plant Cell* 15:63–78
- Alberdi M, Bravo LA, Gutierrez A, Gidekel M, Corcuera LJ (2002) Ecophysiology of Antarctic vascular plants. *Physiol Plant* 115: 479–486
- Bennett MD, Smith JB, Smith RIL (1982) DNA amounts of angiosperms from the Antarctic and South Georgia. *Environ Exp Bot* 22:307–318
- Cardone S, Sawatani P, Rush P, García A, Poggio L, Schrauf G (2009) Karyological studies in *Deschampsia antarctica* Desv. (Poaceae). *Polar Biol* 32:427–433
- Chen W, Provart N, Glazebrook J, Katagiri F, Chang H, Eulgem T, Mauch F, Luan S, Zou G, Whitham S, Budworth P, Tao Y, Xie Z, Chen X, Lam S, Kreps J, Harper J, Si-Ammour A, Mauch-Mani B, Heinlein M, Kobayashi K, Hohn T, Dangl J, Wang X, Zhu T (2002) Expression profile matrix of *Arabidopsis* transcription factor genes suggests their putative functions in response to environmental stresses. *Plant Cell* 14:559–574
- Chew O, Lelean S, John UP, Spangenberg GC (2012) Cold acclimation induces rapid and dynamic changes in freeze tolerance mechanisms in the cryophile *Deschampsia antarctica* E Desv. *Plant Cell Environ* 35:829–837
- Chinnusamy V, Ohta M, Kanrar S, Lee B-H, Hong X, Agarwal M, Zhu J-K (2003) ICE1: a regulator of cold-induced transcriptome and freezing tolerance in *Arabidopsis*. *Genes Dev* 17:1043–1054
- Doherty CJ, Van Buskirk HA, Myers SJ, Thomashow MF (2009) Roles for *Arabidopsis* CAMTA transcription factors in cold-regulated gene expression and freezing tolerance. *Plant Cell* 21:972–984
- Finkelstein RR, Lynch TJ (2000) The *Arabidopsis* abscisic acid response gene *ABI5* encodes a basic leucine zipper transcription factor. *Plant Cell* 12:599–610
- Garg R, Patel RK, Jhanwar S, Priya P, Bhattacharjee A, Yadav G, Bhatia S, Chattopadhyay D, Tyagi AK, Jain M (2011) Gene discovery and tissue-specific transcriptome analysis in chickpea with massively parallel pyrosequencing and web resource development. *Plant Physiol* 156:1661–1678
- Gidekel M, Destefano-Beltran L, Garcia P, Mujica L, Leal P, Cuba M, Fuentes L, Bravo LA, Corcuera LJ, Alberdi M, Concha I, Gutierrez A (2003) Identification and characterization of three novel cold acclimation-responsive genes from the extremophile hair grass *Deschampsia antarctica* Desv. *Extremophiles* 7:459–469
- Giraudat J, Hauge BM, Valon C, Smalle J, Parcy F, Goodman HM (1992) Isolation of the *Arabidopsis* *ABI3* gene by positional cloning. *Plant Cell* 4:1251–1261
- Hu H, Dai M, Yao J, Xiao B, Li X, Zhang Q, Xiong L (2006) Overexpressing a NAM, ATAF, and CUC (NAC) transcription factor enhances drought resistance and salt tolerance in rice. *Proc Natl Acad Sci USA* 103:12987–12992
- Hu H, You J, Fang Y, Zhu X, Qi Z, Xiong L (2008) Characterization of transcription factor gene *SNAC2* conferring cold and salt tolerance in rice. *Plant Mol Biol* 67:169–181
- Jaglo-Ottosen KR, Gilmour SJ, Zarka DG, Schabenberger O, Thomashow MF (1998) *Arabidopsis* *CBF1* overexpression induces *COR* genes and enhances freezing tolerance. *Science* 280:104–106
- Jiang Y, Deyholos M (2006) Comprehensive transcriptional profiling of NaCl-stressed *Arabidopsis* roots reveals novel classes of responsive genes. *BMC Plant Biol* 6:25

- John UP, Polotnianka RM, Sivakumaran KA, Chew O, Mackin L, Kuiper MJ, Talbot JP, Nugent GD, Mautord J, Schrauf GE, Spangenberg GC (2009) Ice recrystallization inhibition proteins (IRIPs) and freeze tolerance in the cryophilic Antarctic hair grass *Deschampsia antarctica* E Desv. *Plant Cell Environ* 32:336–348
- Kasuga M, Liu Q, Miura S, Yamaguchi-Shinozaki K, Shinozaki K (1999) Improving plant drought, salt, and freezing tolerance by gene transfer of a single stress-inducible transcription factor. *Nat Biotechnol* 17:287–291
- Kawaura K, Mochida K, Ogihara Y (2008) Genome-wide analysis for identification of salt-responsive genes in common wheat. *Funct Integr Genomics* 8:277–286
- Kreps JA, Wu Y, Chang H-S, Zhu T, Wang X, Harper JF (2002) Transcriptome changes for *Arabidopsis* in response to salt, osmotic, and cold stress. *Plant Physiol* 130:2129–2141
- Lang D, Weiche B, Timmerhaus G, Richardt S, Riaño-Pachón DM, Corrêa LGG, Reski R, Mueller-Roeber B, Rensing SA (2010) Genome-wide phylogenetic comparative analysis of plant transcriptional regulation: a timeline of loss, gain, expansion, and correlation with complexity. *Genome Biol Evol* 2:488–503
- Lee H, Cho HH, Kim I-C, Yim JH, Lee HK, Lee YK (2008) Expressed sequence tag analysis of Antarctic hairgrass *Deschampsia antarctica* from King George Island, Antarctica. *Mol Cells* 25:258–264
- Lee H, Kim JH, Park M, Kim I-C, Yim JH, Lee HK (2010) Reference genes validation for qPCR normalization in *Deschampsia antarctica* during abiotic stresses. *Antarct Sci* 22:477–484
- Lee J, Noh EK, Choi H-S, Shin SC, Park H, Lee H (2013) Transcriptome sequencing of the Antarctic vascular plant *Deschampsia antarctica* Desv. under abiotic stress. *Planta* 237:823–836
- Lespinet O, Wolf YI, Koonin EV, Aravind L (2002) The role of lineage-specific gene family expansion in the evolution of eukaryotes. *Genome Res* 12:1048–1059
- Leung J, Giraudat J (1998) Abscisic acid signal transduction. *Annu Rev Plant Physiol Plant Mol Biol* 49:199–222
- Lewis Smith RI (1994) Vascular plants as bioindicators of regional warming in Antarctica. *Oecologia* 99(3):322–328
- Libault M, Joshi T, Benedetto VA, Xu D, Udvardi MK, Stacey G (2009) Legume transcription factor genes: what makes legumes so special? *Plant Physiol* 151:991–1001
- Liu Q, Kasuga M, Sakuma Y, Abe H, Miura S, Yamaguchi-Shinozaki K, Shinozaki K (1998) Two transcription factors, DREB1 and DREB2, with an EREBP/AP2 DNA binding domain separate two cellular signal transduction pathways in drought- and low-temperature-responsive gene expression, respectively, in *Arabidopsis*. *Plant Cell* 10:1391–1406
- Lopez-Molina L, Mongrand S, McLachlin DT, Chait BT, Chua N-H (2002) ABI5 acts downstream of ABI3 to execute an ABA-dependent growth arrest during germination. *Plant J* 32:317–328
- Nakamura S, Lynch TJ, Finkelstein RR (2001) Physical interactions between ABA response loci of *Arabidopsis*. *Plant J* 26:627–635
- Nakashima K, Shinwari Z, Sakuma Y, Seki M, Miura S, Shinozaki K, Yamaguchi-Shinozaki K (2000) Organization and expression of two *Arabidopsis* DREB2 genes encoding DRE-binding proteins involved in dehydration- and high-salinity-responsive gene expression. *Plant Mol Biol* 42:657–665
- Nakashima K, Fujita Y, Katsura K, Maruyama K, Narusaka Y, Seki M, Shinozaki K, Yamaguchi-Shinozaki K (2006) Transcriptional regulation of ABI3- and ABA-responsive genes including *RD29B* and *RD29A* in seeds, germinating embryos, and seedlings of *Arabidopsis*. *Plant Mol Biol* 60:51–68
- Nakashima K, Tran LS, Van Nguyen D, Fujita M, Maruyama K, Todaka D, Ito Y, Hayashi N, Shinozaki K, Yamaguchi-Shinozaki K (2007) Functional analysis of a NAC-type transcription factor OsNAC6 involved in abiotic and biotic stress-responsive gene expression in rice. *Plant J* 51(4):617–630
- Rabbani MA, Maruyama K, Abe H, Khan MA, Katsura K, Ito Y, Yoshiwara K, Seki M, Shinozaki K, Yamaguchi-Shinozaki K (2003) Monitoring expression profiles of rice genes under cold, drought, and high-salinity stresses and abscisic acid application using cDNA microarray and RNA gel-blot analyses. *Plant Physiol* 133:1755–1767
- Richardt S, Lang D, Reski R, Frank W, Rensing SA (2007) PlanTAPDB, a phylogeny-based resource of plant transcription-associated proteins. *Plant Physiol* 143:1452–1466
- Riechmann JL, Heard J, Martin G, Reuber L, Jiang C, Keddie J, Adam L, Pineda O, Ratcliffe OJ, Samaha RR, Creelman R, Pilgrim M, Broun P, Zhang JZ, Ghandehari D, Sherman BK, Yu G (2000) *Arabidopsis* transcription factors: genome-wide comparative analysis among eukaryotes. *Science* 290:2105–2110
- Salvucci ME, Crafts-Brandner SJ (2004) Relationship between the heat tolerance of photosynthesis and the thermal stability of rubisco activase in plants from contrasting thermal environments. *Plant Physiol* 134:1460–1470
- Sanchez-Venegas JR, Dinamarca J, Moraga AG, Gidekel M (2009) Molecular characterization of a cDNA encoding Cu/Zn superoxide dismutase from *Deschampsia antarctica* and its expression regulated by cold and UV stresses. *BMC Res Notes* 2:198
- Shinozaki K, Yamaguchi-Shinozaki K (2000) Molecular responses to dehydration and low temperature: differences and cross-talk between two stress signaling pathways. *Curr Opin Plant Biol* 3:217–223
- Shiu S-H, Shih M-C, Li W-H (2005) Transcription factor families have much higher expansion rates in plants than in animals. *Plant Physiol* 139:18–26
- Thomashow MF (1999) Plant cold acclimation: freezing tolerance genes and regulatory mechanisms. *Annu Rev Plant Physiol Plant Mol Biol* 50:571–599
- Tran L-SP, Nakashima K, Sakuma Y, Simpson SD, Fujita Y, Maruyama K, Fujita M, Seki M, Shinozaki K, Yamaguchi-Shinozaki K (2004) Isolation and functional analysis of *Arabidopsis* stress-inducible NAC transcription factors that bind to a drought-responsive cis-element in the early responsive to dehydration stress 1 promoter. *Plant Cell* 16:2481–2498
- Vogel JT, Zarka DG, Van Buskirk HA, Fowler SG, Thomashow MF (2005) Roles of the CBF2 and ZAT12 transcription factors in configuring the low temperature transcriptome of *Arabidopsis*. *Plant J* 41:195–211
- Xiong FS, Ruhland CT, Day TA (1999) Photosynthetic temperature response of the Antarctic vascular plants *Colobanthus quitensis* and *Deschampsia antarctica*. *Physiol Plant* 106:276–286
- Yang Z, Tian L, Latoszek-Green M, Brown D, Wu K (2005) *Arabidopsis* ERF4 is a transcriptional repressor capable of modulating ethylene and abscisic acid responses. *Plant Mol Biol* 58:585–596
- Zhang H, Jin J, Tang L, Zhao Y, Gu X, Gao G, Luo J (2011) PlantTFDB 2.0: update and improvement of the comprehensive plant transcription factor database. *Nucleic Acids Res* 39:D1114–D1117
- Zheng X, Chen B, Lu G, Han B (2009) Overexpression of a NAC transcription factor enhances rice drought and salt tolerance. *Biochem Biophys Res Commun* 379:985–989
- Zhu J-K (2001) Cell signaling under salt, water and cold stresses. *Curr Opin Plant Biol* 4:401–406