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# Identification of a drought responsive gene encoding a nuclear protein involved in drought and freezing stress tolerance in *Arabidopsis*

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## Abstract

Plants have developed adaptive strategies to survive under different abiotic stressors. To identify new components involved in abiotic stress tolerance, we screened unannotated expressed sequence tags (ESTs) and evaluated their cold or drought response in *Arabidopsis*. We identified a drought response gene (*DRG*) encoding a 39.5-kDa polypeptide. This protein was expressed specifically in siliques and was induced by drought stress in most tissues. When a *DRG-GFP* construct was introduced into *Arabidopsis* protoplasts, GFP signals were detected only in the nucleus. The *drg* mutant plant was more sensitive to mannitol-induced osmotic stress in agar plates and to drought or freezing stress in soil than the wild-type. Activating the *DRG* restored the normal sensitivity of *drg* mutants to abiotic stressors. No differences in drought or freezing tolerance were observed between the wild-type and transgenic plants overexpressing the *DRG*. When *DRG* was expressed in a cold-sensitive *Escherichia coli* strain BX04, the transformed bacteria grew faster than the untransformed BXO4 cells under cold stress. These results demonstrate that *DRG* is a nuclear protein induced by abiotic stresses and it is required for drought and freezing tolerance in *Arabidopsis*.

*Additional key words*: *DRG*, *drg* knock out mutant, *Escherichia coli*, mannitol, transgenic plants.

## Introduction

Water availability and extreme temperatures are the two main factors limiting plant growth and yield (Bray *et al*. 2000). Plants have developed adaptive strategies to these types of stressors. Stress tolerance mechanisms are complex and involve a number of biochemical and physiological changes (Ingram and Bartel 1996, Bray *et al*. 2000). Understanding the molecular mechanisms underlying stress tolerance is essential to engineer and develop abiotic stress-tolerant crops.

Appreciable efforts have been made to identify the genes involved in drought stress tolerance mechanisms in plants. Expressed sequence tags (ESTs), suppression subtractive hybridization, and microarray methods have been employed to identify genes associated with plant response to abiotic stressors (Diatchenko *et al*. 1996, Seki *et al*. 2001, Houde *et al*. 2006, Cuming *et al*. 2007, Kim *et al*. 2011). RNA sequencing approaches using new-generation sequencing platforms have also been applied to characterize the changes in gene expression that occur in response to abiotic stressors including water deficit (Mortazavi *et al*. 2008, Qin *et al*. 2008, Rodriguez *et al*. 2010, Huang *et al*. 2012, Choi *et al*. 2013). In addition, the function of some stress-responsive genes has been identified using transgenic plants overexpressing specific genes or using knockout mutants obtained by insertion mutation.

Abiotic stress-responsive genes can be classified into two groups. The first group is transcription factors involved in stress-response gene expression (Yamaguchi-Shinozaki and Shinozaki 2005, Hirayama and Shinozaki

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*Abbreviations*: ABA - abscisic acid; AtNAR - *Arabidopsis thaliana* non-annotated RNA; *DRG* - drought responsive gene; EST - expressed sequence tag; GFP - green fluorescent protein; LEA - late embryogenesis abundant protein; ORF - open reading frame; RT-qPCR - reverse transcription quantitative polymerase chain reaction.
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2010, Nakashima *et al*. 2014). These include abscisic acid (ABA)-responsive transcription factors (AREB/ABF) involved in ABA-dependent gene expression, a dehydration response element binding protein (DREB/CBF), and NAM, ATAF, and CUC transcription factors (Nakashima *et al*. 2014). The drought response transcription factors and their regulatory networks under abiotic stress conditions have been summarized in several reviews (Yamaguchi-Shinozaki and Shinozaki 2005, Hirayma and Shinozaki 2010, Nakashima *et al*. 2014). The second group of drought stress responsive genes are those coding proteins which protect cells under water stress (Ingram and Bartels 1996, Bray *et al*. 2000). These include late embryogenesis abundant (LEA) proteins, molecular chaperones, oxidative stress-related proteins that scavenge reactive oxygen species, and enzymes for lipid and sugar metabolism and biosynthesis of compatible solutes (Vierling 1991, Bray *et al*. 2000, Hundertmark and Hincha 2008). Dehydrins belong to LEA group II and they are typical proteins induced by osmotic stress. The dehydrins are thought to play a role in protecting macromolecules (Close 1996).

Full genome sequencing analyses of higher plants, including *Arabidopsis,* have detected several hundreds of genes upregulated or induced by abiotic stressors (Seki *et al*. 2001, Rodriquez *et al*. 2010). Some of these genes including noncoding RNAs, such as small RNAs, have a significant sequence homology with other known genes deposited in public databases. Identification and functional analyses of these genes will significantly enhance our knowledge of abiotic stress tolerance and resistance mechanisms in plants. We selected a dataset of unannotated ESTs and screened them to determine whether they are involved in cold or drought response in *Arabidopsi*s with the aim to identify different components involved in abiotic stress tolerance.

## Materials and methods

**Plants:** We used *Arabidopsis thaliana* L. ecotype Columbia-0. Seeds were surface sterilized with 70 % ethanol and followed by a 0.05 % (m/v) sodium hypochlorite solution for 10 min and germinated on a solid Murashige and Skoog (MS) medium containing 3 % (m/v) sucrose and 1× Schenk and Hildebrandt (SH) vitamins (*Sigma*, St. Louis, MO, USA) in 9.0-cm Petri dishes. The MS medium was adjusted to pH 5.7, and 1 % (m/v) agar was added to solidify the mixture. In another experiment, seeds were sown in plastic pots containing a 3:1 mixture of potting soil and *Vermiculite*. The plants were grown under a 16-h photoperiod, an irradiance of 33.8 μmol $m^{-2}$ $s^{-1}$, and a temperature of 23 °C.

*Salk_022729* knockout (*DRG* knockout) *Arabidopsis* mutant plants were obtained from the *Arabidopsis Biological Resource Center* (Salk Institute, USA) and germinated on MS medium plates containing 50 μg $cm^{-3}$ kanamycin. The T-DNA insertion in a target gene of knockout plants was verified by gene-specific polymerase chain reaction (PCR) analysis. The homozygous transgenic lines were selected using the Mendelian test on agar plates containing kanamycin and used for further study.

**Identification of *DRG* and sequence analysis:** A total of 294 062 *Arabidopsis* EST sequences were obtained from a public database (*NCBI*, accessed on 2004/7/01) and cleaned using the *Seqclean* program. These EST sequences were mapped onto *Arabidopsis* genes and genome sequences using the *BLAST* program. The ESTs identified to be located within coding sequences, introns, untranslated regions, or promoters were discarded. Finally, 688 ESTs mapped to non-annotated intergenic regions of the *Arabidopsis* genome were selected and called *Arabidopsis thaliana* non-annotated RNAs (AtNARs). Assembly of the ESTs generated 69 contigs, and 317 reads were unique ESTs. We selected 28 of the contigs that included more than 5 reads each and checked expression patterns under normal and drought-stress conditions by reverse transcription-PCR (RT-PCR) analysis (Fig. 1 Suppl. *A*). Two drought stress response transcripts AtNAR01 and AtNAR15 were mapped on putative cDNA candidates between At1g27460 and At1g2747. These cDNA candidates were annotated as At1g27461 and referred to as *DRG* hereafter.

The individual ESTs were searched against the GenBank nucleotide database using *BLASTX.* Multiple sequences were aligned, edited, and amino acid sequence was predicted from the selected ESTs using *Sequencher* (*Gene Codes Corp*., Ann Arbor, MI, USA). The putative molecular masses and pI values of the deduced polypeptides were predicted using *Dnasis Max* (*MiraiBio*, San Bruno, CA, USA). The deduced amino acid sequences were aligned using *Clustal V*.

**Expression of *DRG*:** The total RNA was isolated from plant tissues using an RNeasy plant mini kit (*Qiagen*, Hilden, Germany). First-strand cDNAs were constructed from 2 μg of total RNA by reverse-transcription in a 20 $mm^3$ reaction volume using an oligo$(dT)_{17}$ primer and *Superscript III* reverse transcriptase according to the manufacturer's instructions (*Gibco BRL Life Technologies*, Grand Island, NY, USA). The reactions were conducted at 42 °C for 60 min followed by heating at 70 °C for 5 min. The first-strand cDNA reaction mixtures were diluted by a factor of 5 after which 2 $mm^3$ of diluted cDNA was added to 50 $mm^3$ of a PCR-amplification reaction mixture containing 5 $mm^3$ of a 10× PCR buffer (200 mM Tris-HCl, pH 8.4, 500 mM KCl), 1 $mm^3$ of 10 mM dNTPs, 1 $mm^3$ of each gene-specific primer (10 pM), and 2.5 units of *ExTaq* DNA

polymerase (*Takara Bio*, Shiga, Japan). The PCR reactions were conducted for 28 cycles, each consisting of 30 s at 95 °C, 30 s at 58 to 64 °C, 90 s at 72 °C, and 5 min of termination at 72 °C. We used the following gene-specific forward and reverse primers: F: 5′-AAGTCTATCTGCGGTCAGTGGT-3′ and R: 5′-GTGATGACTGAGAACAATGGGT-3′ for *AtNAR01*; F: 5′-TCAGCCAATCTCCTTACCTTCG-3′ and R: 5′-ACCATTCTCAAAACCGTAGCCA-3′ for *AtNAR15*; F: 5′-GAGCACCTCATCATGGACAG-3′ and R: 5′-AGGTTGTGGATAGCGTCCTG-3′ for *AtNAR27*; F: 5′-CGTGTTGAAGAGAAAGAAGGCAT-3′ and R: 5′-AGAGCAAGAACCAAATGAAGAACA-3′ for *ubiquitin*. The PCR products were separated on 1 % (m/v) agarose gels and stained with ethidium bromide to visualize bands. The RT-qPCR was carried out using a *Rotor-Gene RG-3000* cycler (*Corbett*, Sydney, Queensland, Australia) using a *QuantiTect SYBR Green* PCR kit (*Qiagen*) according to the manufacturer's instructions. The RT-qPCR program consisted of pre-denaturation at 95 °C for 10 min and 40 amplification cycles at 95 °C for 15 s, 60 °C for 30 s, and 72 °C for 30 s. All samples were run in duplicate and an n-fold differential expression was calculated using the comparative Ct method (the n-fold differential expression was calculated by $2^{-\Delta\Delta Ct}$).

**Localization of DRG:** The *DRG* coding region was amplified by PCR using *ExTaq* DNA polymerase (*Takara Bio*) to identify the cellular location of the DRG protein. Gene specific primers used for the PCR were F: 5′-GTCGACATGATTAAGATACTCAAC-3′ and R: 5′-CTGCAGCTATCTGCGGTCAGTGGT-3′. The full open reading frame (ORF) sequence of the *DRG* cDNA was fused upstream of a reporter gene encoding the green fluorescent protein (GFP) in the p326-CaMV35S-*GFP* plant expression vector (p326-*GFP*) (Kim *et al*. 2011). The p326-35S-*DRG-GFP* recombinant vector (p326-*DRG-GFP*) was subjected to a transient assay using *Arabidopsis* protoplasts. The protoplasts were examined under a fluorescent microscope (*Axioskop*, *Carl Zeiss*, Jena, Germany) using a fluorescein isothiocyanate set (excitation at 450 - 490 nm; a dichromic mirror at 510 nm, and a band-pass filter at 515 - 561 nm) to detect a GFP expression.

**Cold shock test in *Escherichia coli*:** The *DRG* cDNA coding region was prepared by PCR with the following gene specific primers containing a restriction enzyme site: F: 5′-CATATGATTAAGATACTCAACCCCC-3′ and R: 5′-GGATCCAGTCTATCTGCGGTCAGTGGT-3′ and subcloned into the *Nde*I/*Bam*HI site of the pINIII vector (Kim *et al*. 2008). The *DRG* coding regions and junction sequences were confirmed by DNA sequencing. The pINIII expression vectors containing either the *DRG* or the gene coding cold shock protein A were introduced into *Escherichia coli* strain BX04, which is a cold-sensitive mutant, and used for the cold treatment (17 °C for 5 d) test (Kim *et al*. 2008). The pINIII vector without a DNA insert was used as control. The transformed BX04 cells were grown in a Luria-Bertani (LB) medium containing ampicillin and kanamycin. Isopropyl-D-thiogalactopyranoside (IPTG; 0.2 mM) was added and the bacterial cultures were incubated at low temperatures of 17 °C for 4 h. Growth of the BX04 cells expressing each gene was measured by spotting the diluted cultures (a serial dilution of the cultures, initially at $A_{600}$ = 0.5, from $10^{-1}$ to $10^{-5}$) on LB-agar plates containing IPTG and incubating at 37 and 17 °C, respectively.

**Generation of transgenic *Arabidopsis* plants:** The *DRG* coding region was amplified by PCR using gene-specific primers (F: 5′-GTCGACATGATTAAGAT ACTCAAC-3′ and R: 5′-CTGCAGCTATCTGCGGTC AGTGGT-3′) containing *Sal*I and *Pst*I sites and was introduced into the pPZP211-EX vector under the control of the 35S promoter to generate *DRG* overexpressing *Arabidopsis* plants. The recombinant vector was introduced into *Agrobacterium tumefaciens* L. GV3101 cells. *Arabidopsis* was transformed as described previously (Ha *et al*. 2007). The transgenic plants obtained by introducing the *DRG* into wild-type and *drg* plants were named *DRG*-OX and *drg*-OX, respectively. The seeds were surface-sterilized as described in previous section and germinated on MS agar plates containing 50 µg $cm^{-3}$ kanamycin to select the transgenic plants. The homozygous transgenic line was isolated using the Mendelian test on MS agar plates containing kanamycin (50 µg $cm^{-3}$) and using *DRG*-specific PCR analysis. The *Arabidopsis* genomic DNA was isolated from green leaves using a DNeasy plant mini kit (*Qiagen*) and used as PCR template.

**Abiotic stress tolerance test:** The 7-d-old seedlings were transferred to a fresh medium with or without 50 to 300 mM mannitol to evaluate osmotic stress tolerance. The seedlings were allowed to grow vertically along with the controls. After two weeks, the seedlings were photographed, and root length was measured. All experiments were performed in triplicate. Wild-type, *drg*, *drg*-OX, and *DRG*-OX plant seeds were sown in plastic pots containing soil and incubated in a growth chamber under the same conditions. Three-week-old plants were held at -8 °C for 3 or 6 h to evaluate freezing tolerance, and then transferred back to the growth chamber under normal conditions to determine survival rate. We stopped watering the three-week-old plants in the pots for two weeks to evaluate drought tolerance. Then, the plants were watered and allowed to recover for 3 d before assessing survival rate. All experiments were performed in triplicate.

## Results and discussion

The EST analysis helped identify the *DRG* transcript. The *DRG* (At1g27461) encoded a basic polypeptide consisting of 354 amino acids (39.5 kDa, pI 9.45). Arginine (9.49 mol%) and serine (9.03 mol%) were the most abundant amino acids in the polypeptide. No putative conserved domains were detected in the DRG. Fig. 1 shows the amino acid sequence alignment of the DRG and homologs from *Rincinus communis* (accession No. XP_002510084), *Vitis vinifera* (No. XP_002283353), and *Populus trichocarpa* (No. XP_002320025). Homologs of DRG were also found in a fern *Selaginella moellendorffii* (No. XP_002969485) and a moss *Physcomitrella patens* (No. XP_001763429). However, no DRG homologs were detected in green algae species. We constructed a local database including a genomic DNA and transcriptome sequences from red algae including *Pyropia* (Choi *et al*. 2013) and searched for *DRG* homologs. However, no *DRG* homologs were detected in this database suggesting that the *DRG* is limited to embryophytes.

We extracted the total RNA content from roots, stems, leaves, flowers, and siliques from soil-grown *Arabidopsis* to evaluate *DRG* expression pattern. The *DRG* transcripts were detected in all the organs. In particular, the *DRG* transcripts were abundant in siliques (Fig, 2*A*). These results suggest that *DRG* was highly expressed in siliques and upregulated by the drought stress. We checked *DRG* expression pattern in leaves under the drought stress (Fig. 2*B*). The *DRG* responded quickly to the water stress and increased about 100-fold after 12 h of the drought stress. These results suggest that the *DRG* is expressed in dehydrated tissues, such as seeds, and upregulated in leaf tissues by drought stress.

The sequence analyses showed the presence of putative *cis*-acting elements, such as ABRE, DRE, and MYC, involved in abiotic stress expression in the *DRG* promoter (Fig. 1 Suppl.). The presence of these *cis*-acting elements suggests that the *DRG* is induced or up-regulated by transcription factors, such as ABF or CBF, under abiotic stresses (Shinozaki and Yamaguchi-Shinozaki 2000, Hirayama and Shinozaki 2010).

We examined the *DRG* for its ability to complement

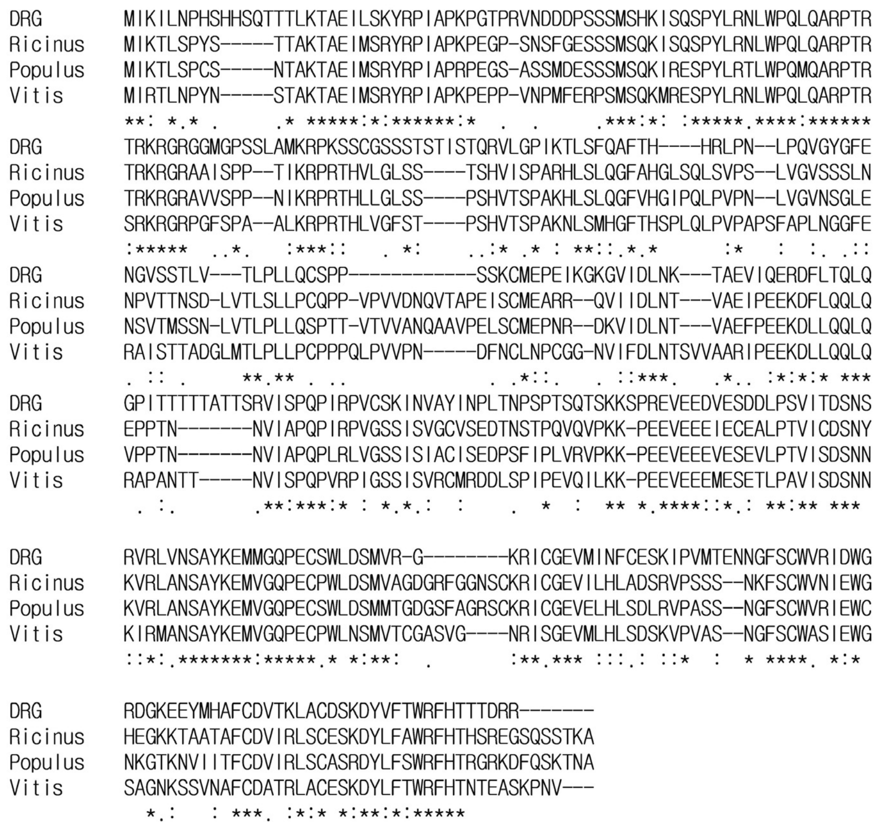


Fig. 1. The comparison of amino acid sequences of polypeptides coded by the drought responsive gene. Amino acid residues are designated as single-letter codes. *Asterisks* and *periods* indicate identical and homologous amino acid sequences, respectively. *Dashes* indicate sites where the sequence has been expanded to allow for an optimum sequence alignment. The alignments of the deduced amino acid sequences were conducted using *CLUSTAL V*.

defects in growth of the *E. coli* cold-sensitive strain BXO4 at the low temperature as described by Kim *et al*. (2008) with the aim to understand the functional role of the *DRG* during cold stress. The colony-forming ability of the BXO4 cells transformed with the *DRG* was assessed on LB plates at 17 °C. The BXO4 cells transformed with the pINIII empty vector or *CspA* were used as negative and positive controls, respectively. Expression of the introduced *DRG* and *CspA* gene was confirmed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (Fig. 3*A*). The BXO4 cells harboring the pINIII-*DRG* or pINIII-*CspA* constructs grew well at 37 °C (Fig. 3*B*). However, the growth rate of BXO4 cells expressing either the *DRG* or *CspA* gene was much higher than that of the control cells containing only the pINIII vector when the cells were subjected to 17 °C (Fig. 3*B*). These results suggest that the *Arabidopsis DRG* suppressed cold sensitivity in the *E. coli* cells at the low temperature and played a role during the cold acclimation.

We obtained the Salk_022729 *drg* knockout mutant. The Salk_022729 mutants had the T-DNA at 730 and

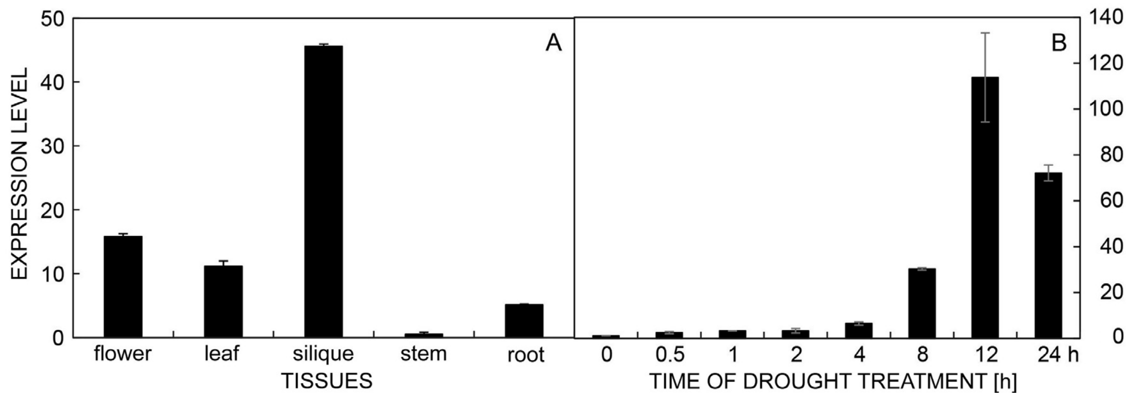


Fig. 2. Expression patterns of the drought responsive gene (*DRG*). *A* - *DRG* expression in leaves, stems, roots, flowers, and siliques. Plant tissues were snap frozen and stored at -80°C until use. The total RNA was isolated from harvested tissues and reverse-transcribed using oligo-dT primers. *B* - Soil-grown plants were uprooted and placed on paper towels to induce a drought stress. Leaves were harvested at indicated time points. Complementary deoxyribonucleic acids were amplified using gene-specific primer sets. The *ubiquitin* gene was used as internal control. Means ± SD, *n* = 3.

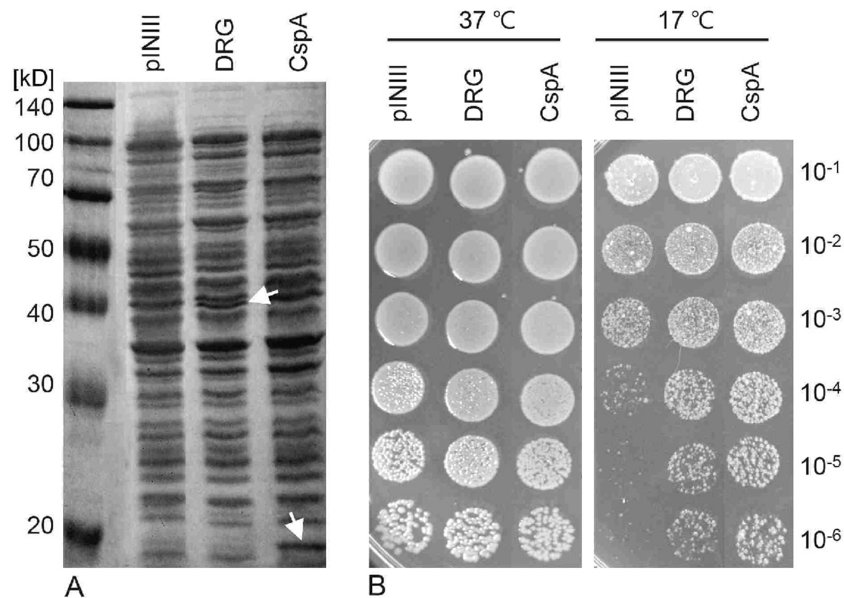


Fig. 3. The effect of the drought responsive gene (*DRG*) on growth of *E. coli* during cold shock. *A* - Expression of *DRG* or a gene for cold shock protein A (CspA) in BX04 cells was verified by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Bands corresponding to each protein are indicated by *arrows*. *B* - Growth of BX04 cells expressing each gene was measured by spotting diluted cultures from primary cultures at $A_{600}$ = 0.5 on LB-agar plates, and the cells were incubated at 37 and 17 °C, respectively. The photographs were taken after 1 d for the cells incubated at 37 °C and after 5 d for the cells incubated at 17 °C. pINIII - empty vector as control.

731 bp from the *DRG* initiation codon (Fig. 2 Suppl.). We introduced the *DRG* into the *drg* mutant (*drg*-OX) and wild-type (*DRG*-OX) *Arabidopsis* plants to overexpress the *DRG* in plants. The transgenic plants were confirmed by the integration and expression of the *DRG* using the PCR and RT-PCR analyses (Fig. 3 Suppl.).

The seeds of the wild-type (Col-0), mutant (*drg*), and transgenic plants (*drg*-OX and *DRG*-OX) were germinated on MS agar plates to confirm if the *DRG* is involved in water stress tolerance in *Arabidopsis*. The seven-d-old seedlings obtained were transferred to MS agar plates containing 50 - 300 mM mannitol, and root growth of each plant was inspected after two weeks (Fig. 4*A*). No significant differences in phenotype were found between the wild-type and *drg* mutant plants on MS agar plates without mannitol. Root growth was reduced by 100 mM mannitol and suppressed markedly by > 200 mM mannitol. The *drg* seedlings were more sensitive to mannitol than the wild-type. The sensitivity of *drg* to osmotic stress caused by mannitol was rescued by introducing and expressing the *DRG* (*drg*-OX). These results demonstrate that the *DRG* may play an important role in osmotic stress tolerance.

We evaluated the survival rates of the wild-type *Arabidopsis* (Col-0), *drg*, *DRG*-OX, and *drg*-OX plants in pots with soil to determine the function of the *DRG* in drought stress tolerance. We transplanted three-week-old plants into pots and stopped watering them for two weeks. The plants were allowed to recover for 3 d after they were watered again. All plants performed equally well under the well water supply, however, the survival rate of the *drg* plants was much lower than that of the wild-type plants under the drought stress (Fig. 4*B*). The sensitivity of *drg* to the drought stress was rescued by introducing and expressing the *DRG* (*drg*-OX), similarly as under the osmotic stress caused by the mannitol treatment (Fig. 4*B*). These results demonstrate that the *DRG* is involved in drought stress tolerance in *Arabidopsis*. No significant difference in drought tolerance was observed between the wild-type and *DRG*-OX plants. These results suggest that the *DRG* was

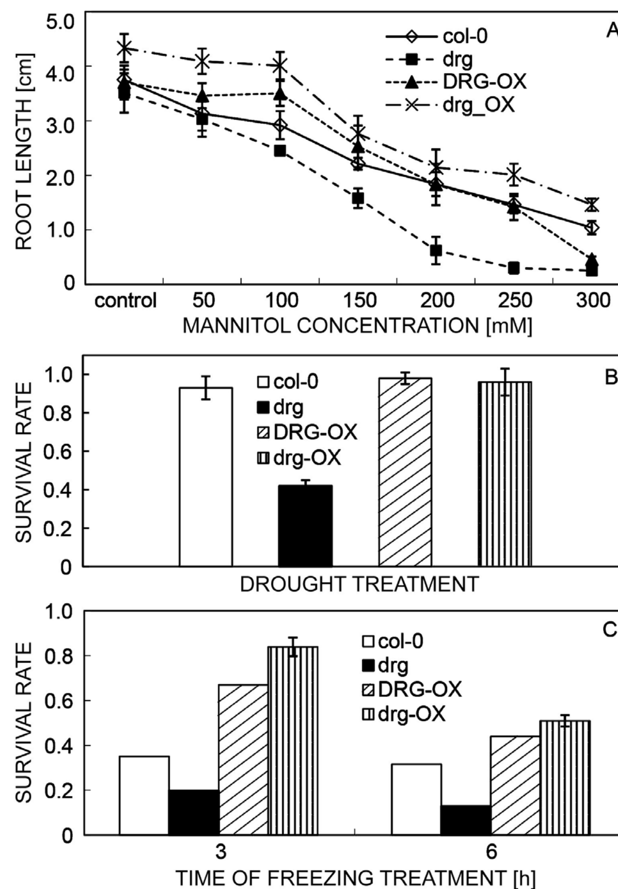


Fig. 4. The drought responsive gene (*DRG*) plays a role in abiotic stress tolerance in plants. *A* - 7-d-old seedlings were transferred to MS agar plates containing 50 - 300 mM mannitol, and root growth was assessed after two weeks. *B* - Watering 3-week-old plants was stopped for two weeks and then the plants were allowed to recover for 3 d. *C* - 3-week-old plants were exposed to -8 °C for 3 or 6 h, moved back to normal growth conditions, and survival was assessed one week later. col-0 - *Arabidopsis thaliana* ecotype col-0, drg - *DRG* knock out mutant, DRG-OX - col-0 transformant over-expressing *DRG* gene, drg-OX - drg transformant over-expressing *DRG* gene. Means ± SD, $n = 3$.

required for drought tolerance and that the *DRG* expression in the wild-type plants under the drought conditions was sufficient. Thus, over-expressing the *DRG* was not required to increase stress tolerance.

The three-week-old wild-type and transgenic plants were exposed to -8 °C for 3 or 6 h to test their freezing tolerance. After the exposure, the plants were moved back to the normal growing conditions and the number of surviving plants was counted one week later. The *drg* plants were much more sensitive to the freezing stress than the wild-type plants (Fig. 4*C*). The sensitivity of the *drg* plants to the freezing stress was recovered by introducing and overexpressing the *DRG* (*drg*-OX). These results demonstrate that the *DRG* played a role in freezing stress tolerance in *Arabidopsis*. The survival rates were much lower when the plants were exposed to the freezing conditions for 6 h than for 3 h. The relative survival rates of the *drg*-OX and DRG-OX transgenic plants were significantly lower than those of the wild-type plants after the longer freezing stress exposure (6 h). The survival rate of transgenic plants harboring the overexpressed *DRG* was much higher than that of the wild-type plants and *drg* mutant. These results suggest that loss of *DRG* function had a significant negative effect on drought and freezing stress tolerance and that overexpressing the *DRG* increased freezing tolerance in *Arabidopsis*.

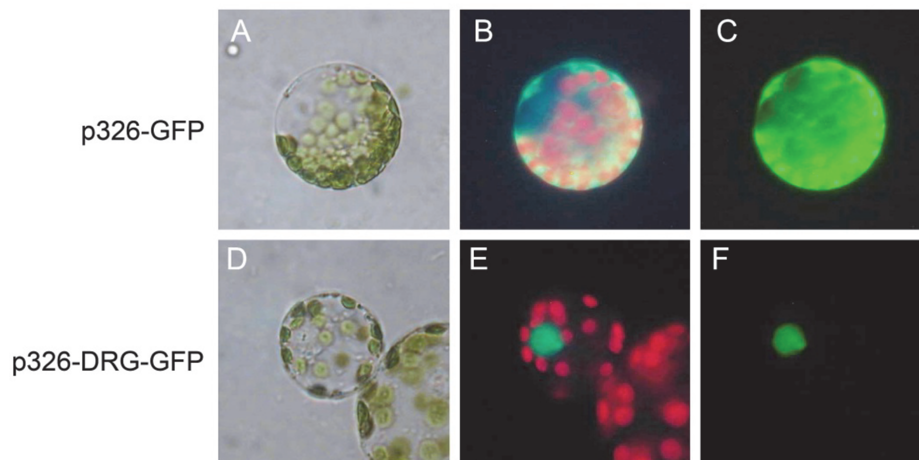


Fig. 5. Nuclear localization of the protein of the *DRG* in *Arabidopsis* protoplasts. A reporter gene encoding a green fluorescent protein (GFP) was fused to the *DRG* under control of the CaMV 35S promoter to determine cellular location of the protein. The constructs were introduced into *Arabidopsis* protoplasts. The 326-GFP vector served as control. Individual cells were observed for the protein location by GFP fluorescence after overnight induction. *A* and *D* - An interference contrast image for the entire cell structure; *B* and *E* - a red-filtered cell image; *C* and *F* - a green-filtered cell image. p326-GFP - empty vector with GFP as control, p326-DRG-GFP - DRG-GFP recombinant vector.

The sequence homology analysis shows that no known domain or signal peptide was detected in the polypeptide originated by *DRG* expression. The full ORF sequence of the *DRG* cDNA was fused upstream of a reporter gene encoding the GFP and subjected to a transient assay using *Arabidopsis* protoplasts to identify the location of the *DRG* product in cells. The p326-*GFP* vector revealed GFP signals in the cytoplasm and nucleus (Fig. 5*A-C*), whereas the p326-*DRG-GFP* vector showed GFP signals only in the nucleus (Fig. 5*D-F*). These results suggest that DGR was located in the *Arabidopsis* nucleus. Many proteins involved in chromatin maintenance and gene expression are located in the nucleus; however, no known protein domains, such as DNA- or RNA-binding domains, were detected in the DGR. Therefore, the DGR may not be involved in gene expression but may play a role in osmotic stress tolerance in a similar way as LEA proteins in the nucleus. Several studies have identified genes involved in abiotic stress tolerance but they encode unknown functions. For example, *Cor15a* encodes a polypeptide of an unknown function but affects freezing tolerance (Artus *et al*. 1996). A high temperature response gene *HTR2* from marine red algae encodes a novel nuclear protein and is involved in heat tolerance in *Chlamydomonas* (Kim *et al*. 2011). Although genome information helps detect expression of stress response in all predicted genes in a genome using RNA sequencing or microarrays, the biological functions of many genes remain unknown. Our results contribute to better understanding of *DRG* function in *Arabidopsis* and of stress response mechanisms in plants.

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