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Research Article

Members of the ran family of stress-inducible small GTP-binding proteins are differentially regulated in sweetpotato plants

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Abstract Ran is a small GTP-binding protein that binds and subsequently hydrolyzes GTP. The functions of Ran in nuclear transport and mitotic progression are well conserved in plants and animals. In animal cells, stress treatments cause Ran relocalization and slowing of nuclear transport, but the role of Ran proteins in plant cells exposed to stress is still unclear. We have therefore compared Ran genes from three EST libraries construed from different cell types of sweetpotato and the distribution pattern of Ran ESTs differed according to cell type. We further characterized two *IbRan* genes. *IbRan1* is a specific EST to the suspension cells and leaf libraries, and *IbRan2* is specific EST to the root library. *IbRan1* showed 94.6 % identity with *IbRan2* at the amino acid level, but the C-terminal region of *IbRan1* differed from that of *IbRan2*. These two genes showed tissue-specific differential regulation in wounded tissues. Chilling stress induced a similar expression pattern in both *IbRan* genes in the leaves and petioles, but they were differently regulated in the roots. Hydrogen peroxide treatment highly stimulated *IbRan2* mRNA expression in the leaves and petioles, but had no significant effect on *IbRan1* gene expression. These results showed that the transcription of these two *IbRan* genes responds differentially to abiotic stresses and that they are subjected to tissue-specific regulation. Plant Ran-type small G-proteins are a multigenic family, and the characterization of each *Ran* genes under various environmental stresses will contribute toward our understanding of the distinctive function of each plant Ran isoform.

Keywords EST analysis, Tissue-specific expression, Wounding, Chilling, Hydrogen peroxide

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Introduction

GTP-binding proteins constitute a superfamily of proteins that posses structurally preserved GTP-binding domain. They are structurally and functionally classified into at least five families (Ras, Rho, Rab, Arf/Sar1, and Ran families) and regulate a wide variety of cell functions, such as signal transduction, cell proliferation, cytoskeletal organization, intracellular membrane trafficking, and gene expression. Small GTP-binding proteins are numeric G proteins with a molecular mass of between 20 and 40 kDa. In contrast to other small GTP-binding proteins such as the Ras and Rho families, Ran protein is not membrane bound. Ran proteins play key roles in transporting RNA and proteins through the nuclear pores, controlling nuclear processes, and mediating the assembly of the nuclear envelope and spindle during the cell mitotic cycle (Görlich and Kutay 1999; Clarke and Zhang 2001; Hetzer et al. 2002; Ciciarello et al. 2007). The GTP-bound Ran (RanGTP) and GDP-bound Ran (RanGDP) have different functions in nuclear transport, and their respective abundance is regulated by the spatial separation of RanGAP and RanBP1 (Ran binding protein 1), both of which are largely in cytoplasm, and by RCC1 (RanGEF; regulator of chromosome condensation 1), which is a chromatin-associated nuclear protein. The asymmetric distribution of nucleotide exchange and hydrolysis enzymes across the nuclear envelope suggests that RanGTP should be largely in nucleus and RanGDP largely in cytoplasm. This distribution plays a key role in determining the directionality of nuclear transport, i.e., the entry and exit of molecules from the cell nucleus.

It has been shown that there is a cross-talk between the respective signaling pathways for nuclear transport and stress. Nucleocytoplasmic distribution of proteins changes in the stressed cells. In mammalian cells, exposure to severe cellular stresses (e.g., oxidative stress, heat shock, UV irradiation, hyperosmotic stress) induces the collapse

of the Ran distribution in the cytoplasm and nuclear compartment, thereby inhibiting classical nuclear protein import (Miyamoto et al. 2004; Kelly and Paschal 2007). Stress also decreases the availability of RanGTP and induces the degradation of Ran, importin-β, and Nup153 (Kodiha et al. 2004). In yeast, oxidative stress has been shown to reduce NLS-dependent import (Stochaj et al. 2000) and hyper-osmotic stress to cause the delocalization of nuclear proteins (Nanduri and Tartakoff 2001). Novel aspects of Ran proteins have been recently reported in shrimp where it plays important roles in the innate immunity system through regulating the process of hemocytic phagocytosis by interaction with myosin (Han and Zang 2007; Liu et al. 2009).

The various functions of Ran signaling in nuclear transport and mitotic progression are well conserved in plants and animals. Overexpression of plant Ran genes suppresses the phenotype of the cell cycle regulatory mutant *pim1-46* fission yeast (Ach and Gruissem 1994; Merkle et al. 1994). Transgenic plants overexpressing wheat *RAN1* (*TaRAN1*) have an elevated mitotic index and prolonged life cycle (Wang et al. 2006). *In vitro* studies have revealed an interaction between *Arabidopsis* Ran and RanBP1 (Haizel et al. 1997), as well as functional role of AtRanBP1c as a co-activator of RanGAP. *RanGAP* genes from *Medicago sativa* and *Arabidopsis* can complement the yeast RanGAP mutant *rna1* (Pay et al. 2002).

Despite these similarities, there are a number of fundamental distinct differences between the plant Ran system and its mammalian counterpart (Ma 2007; Meier 2007). Plant Ran is a multigene protein family (Vernoud et al. 2003), while the genome of mammalian and *Cenorabditis elegans* each contains a single Ran gene. Four Ran genes have been identified in *Arabidopsis*, of which three, *AtRan1*, *AtRan2*, and *AtRan3*, are highly homologous (95-96% identity), differing only in their C-terminal regions (Haizel et al. 1997). AtRan3 is targeted into the nucleus, similar to its animal counterpart (Yano et al. 2006), while AtRan2 localizes to the perinuclear region and nuclear envelope and is absent inside the nucleus (Ma et al. 2007). These two Ran proteins differ in their subcellular localizations and, more importantly, in their biological functions. *PsRan1* gene expression in *Arabidopsis* and pea is differentially regulated by light sources and phytochrome-mediated signaling pathways (Lee et al. 2008). Two tobacco genes, *NtRan A1* and *B1*, are highly expressed in the root and stem, but not expressed at all in meristematic tissues and differently expressed in the stamen. There have also been several reports of plants and vertebrates using different mechanisms to target RanGAP to the nuclear envelope (Mahajan et al. 1997; Jeong et al. 2005).

In plants, Ran and RanBP together with the nucleoporins are considered to be involved in a variety of cellular response pathways, including those for regulating hormone sensitivities, light signaling, and pathogen resistance (Meier 2007; Xu and Meier 2007; Zhao et al. 2007; Tameling and Baulcombe 2007). The reduced expression of the OsRAN2 transcript was demonstrated with salt and osmotic treatment (Zang et al. 2010). AtRanBP1c has been reported to be a critical factor in root growth and development (Kim et al. 2001). NbRanBP1-silenced plants exhibit stress responses, such as reduced mitochondrial membrane potential, excessive production of reactive oxygen species, and induction of defense-related genes (Cho et al. 2008). However, the functions of Ran protein in plant signaling pathways involved in the response to environmental stimuli remain poorly defined. In addition, the transcriptional regulation of plant Ran genes has still to be addressed.

Here sweet potato (*Ipomoea babatas*) *Ran* genes were compared in three EST libraries constructed from different cell types (suspension cultured cells, early storage roots, and leaves). We found that the transcript level and isoform of the *Ran* gene were different in these three EST libraries. We also isolated two *IbRan* genes and investigated their transcriptional regulation in tissues after exposure to various stresses (hydrogen peroxide, wounding, and chilling treatments). The two sweet potato *Ran* genes showed different transcriptional regulation in response to various environmental stresses with tissue-specificity.

Materials and methods

Plant materials

Sweet potato plants (*Ipomoea batatas* cv. Yulmi) were maintained in a pot inside the greenhouse under 16 h light/8 h dark (25℃) cycles. Three-week-old propagated plants, or parts thereof, were used for stress treatments.

Southern blot analysis

Whole cells of sweetpotato cultured in cell suspension were frozen and ground to a fine powder using liquid nitrogen, and genomic DNA was isolated according to the manufacturer's instructions (QIAGEN, Germany). The genomic DNA (10 μg) was then digested with *Eco*RI and *Hind*III independently, followed by electrophoretic separa-

tion in a 0.8% agarose gel. These restriction enzymes do not have any recognition sequences within the *IbRan1* cDNA sequence. After a complete denaturation and subsequent renaturation, the gel was blotted onto a positively charged nylon membrane (Hybond-N^+, Amersham Biosciences, UK). Biotin (biotin-14-dCTP, Invitrogen, USA) was used as a probe and labeled by PCR amplification using *IbRan1* cDNA as a template. The PCR analysis was performed in a 20 μl volume containing 1.25 U *ExTaq* DNA polymerase (Takara, Japan), 2 μl of 10 × *Taq* buffer, 4 μl of 5 × dNTPs mix (0.25 mM biotin-14-dCTP, 0.25 mM dCTP, 0.5 mM dATP, 0.5 mM dGTP and 0.5 mM dTTP), and 10 pmole of T3 and T7 primers. The PCR cycling conditions consisted of 94℃ for 5 min, 30 cycles of 30 s at 94℃, 30 s at 60℃, and 1 min at 72℃, with a final extension step of 7 min at 72℃. The labeled probe was purified using a PCR Purification kit (QIAGEN). The membrane was hybridized at 65℃ for 16 h and then washed twice with 2 × SSC/1% SDS at room temperature for 10 min, twice with 1 × SSC/1% SDS at room temperature for 20 min, and twice with 1 × SSC at room temperature for 10 min. Hybridized signals were detected using the Southern-Star™ System (Tropix, USA).

Total RNA isolation and RT-PCR analysis

One gram of plant tissues was ground in liquid nitrogen and homogenized in the extraction buffer (0.25 M Tris-Cl, pH 9.0, 0.25 M NaCl, 0.05 M EDTA, 27 mM naphthalene disulfonic acid, 0.25 M *p*-aminosalicylic acid) containing 0.7 M β-mercaptoethanol and phenol. The solution was extracted first with chloroform, and then with phenol: chloroform:isoamyl alcohol (25:24:1). Nucleic acids in aqueous phase were isopropanol-precipitated. The pellet was washed, dried at room temperature and resuspended in 1 ml TE buffer. RNA was precipitated with 2 M LiCl overnight at 4℃. After centrifugation, the pellet was washed with 70% ethanol, dried at room temperature, and resuspended in TE buffer. The RNA was stored at -70℃ until use. Semi-quantitative RT-PCR was performed to analyze the expression pattern of *IbRan1* and *IbRan2*. Two μg of total RNA was utilized for the synthesis of first strand cDNA. Reverse transcription was performed using oligo $(dT)_{20}$ (Invitrogen, USA) as a primer and Superscript™ II reverse transcriptase (Invitrogen). Aliquots consisting of one-tenth of the RT product were used as a template for each of the following PCR amplification. The PCR cycling conditions for *IbRan1* and *IbRan2* consisted of 94℃ for 3 min, 30 cycles of 30 s at 94℃, 1 min at 60℃, and 1 min at 72℃, with a final extension step of 7 min at 72℃. Specific primers for *IbRan1* are Ran1F (5'-C CTA ACC AAG CAA CGA CG-') and Ran1R (5' -AAGTACTC GTGTAAGTACC-3'). Specific primers for *IbRan2* are 46F115'UTR (5'-TCCTCA ATCCACCTCTCTC-3') and 46F11R1 (5'- CACGAGATGTGCAGAAGAC-3')

Stress treatments

Sweet potato plants were grown in the greenhouse at 25℃ for 3 weeks and then exposed to abiotic stresses. For cold stress, sweetpotato plants in a pot were exposed at 15℃ for 24, 48 and 72 h. For H_2O_2 treatment, the second and third leaves from the top were removed from the plant and incubated in 440 mM H_2O_2 solution at 25℃ for 12 and 24 h. Sterile water was used as a control for the H_2O_2 treatment. Wounding stress was performed according to Sasaki et al. (2002). Fully expanded leaves were detached and immediately cut into pieces with a razor blade. After removing the midrib, we placed the leaf pieces (six per leaf) on moistened filter paper with distilled water and incubated the leaf pieces for 1, 4, 8, 12 and 24 h at 25℃ under continuous illumination. Untreated and stress treated plant samples were collected, frozen in liquid nitrogen, and stored at -70℃ for RNA isolation.

Results

Isolation and sequencing of *IbRan* cDNAs from three EST libraries

In a previous study we constructed an EST library from sweet potato suspension-cultured cells (S-library) and compared the 1,411 ESTs of the library with the 2,859 ESTs from a sweet potato early storage roots library (R-library) and the 1,079 ESTs of a sweet potato leaf library (L-library) (You et al. 2003; Kim et al. 2006). Most abundant ESTs in the S-library were very low or absent in the L- and/or R-libraries. *Ran* gene was one of the highly abundant ESTs in suspension-cultured cells. As plant Ran proteins are considered to be involved in the regulation of various physiological responses, we compared the Ran transcripts in these three EST libraries and found that three Ran ESTs were present in the S-library and one Ran EST was present in each of the R- and L-libraries (Table 1). Partial sequences of the three Ran ESTs (CO500988, CO500700, and CO500894) from the S-library and one Ran EST (CB329957) from the L-library showed 97-98 % identity

at the nucleotide level. These four Ran ESTs showed about 77% identity with the root Ran EST (BU692833) at the nucleotide level.

To investigate the structural characteristics and transcriptional regulation of *Ran* genes, we attempted to obtain full-length Ran cDNAs. First, the full length of three Ran cDNAs from the S-library was sequenced and found to have 98-99% identity at the nucleotide level. Two of these showed 100% identity at the amino acid level and the other differed by one amino acid residue, indicating that they may have originated from one ancestor gene. We denoted one of these Ran ESTs (CO500988) as *IbRan1*. The cDNA of the *IbRan1* gene consists of 1,059 nucleotides. This gene encodes a polypeptide of 221 amino acids, with 49 bp of 5'-untranslated region (UTR) and 288 bp of 3'-UTR. The putative polyadenylation signal, TATAAAT, was found to be located 79 bases upstream from the start of the poly $(A)^+$ tail. *IbRan1* showed 100 % identity at the amino acid level and 99 % identity at the nucleotide level with the full-length Ran EST (CB329957) from the L-library.

A partial root Ran EST (BU692833) was denoted as *IbRan2*. The length of the partial *IbRan2* cDNA is 472 bp, which encodes 70 amino acids. The putative polyadenylation signal, ATATTA, was found to be located 27 bases upstream from the start of the poly $(A)^+$ tail. The sequences of 3'-UTR of *IbRan2* did not show any significant similarity with that of *IbRan1*. To isolate the full-length of *IbRan2* cDNA, we designed several sets of specific primers in the 3'-UTR of *IbRan2* gene and performed PCR using the R-library as a template; however, we failed to obtain the full-length of *IbRan2* cDNA. In contrast, genomic walking PCR using specific primers of the partial *IbRan2* cDNA

Table 1 Expression pattern of Ran expressed sequence tags (ESTs) among the three EST libraries constructed from sweetpotato suspension cells (S), roots (R), and leaves (L).

Gene	EST library	EST GenBank Acc.	Identity in EST sequences
Run	S	CO500988	100%
		CO500700	98%
		CO500894	97%
	L	CB329957	98%
	R	BU692833	77%

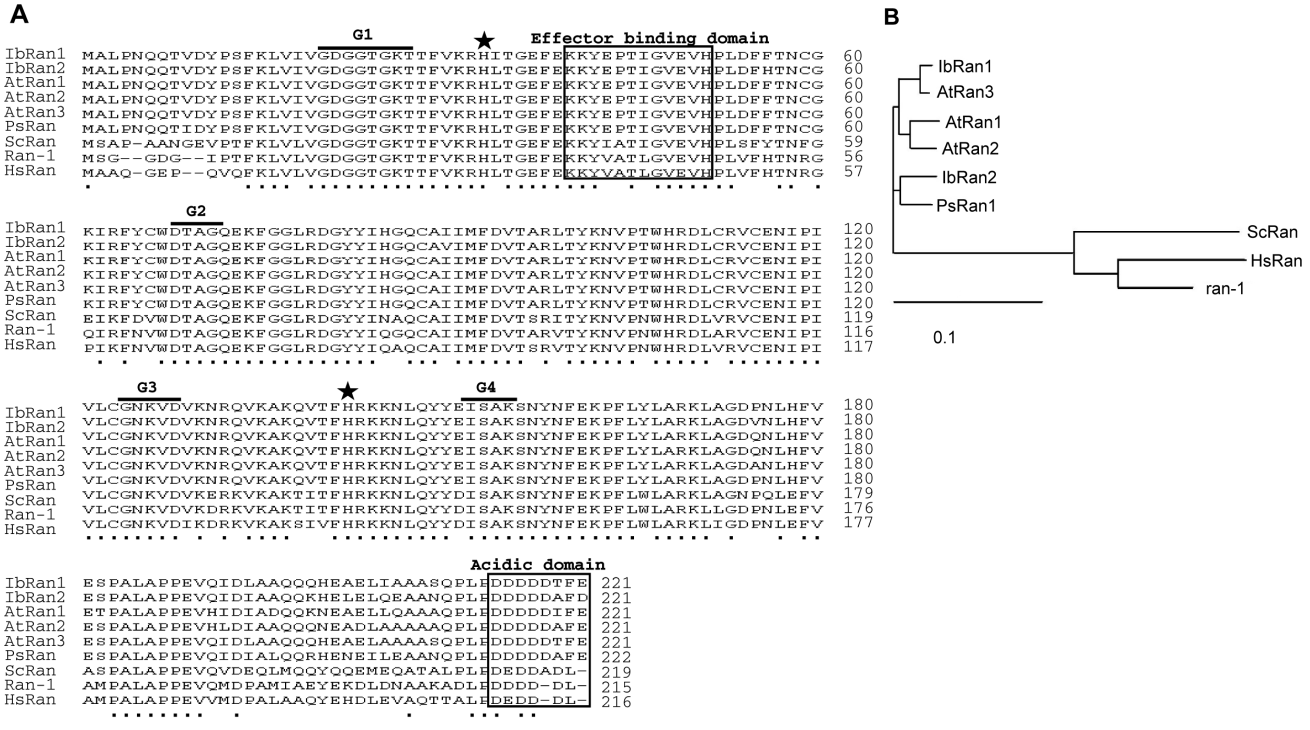

Fig. 1 Sequence comparison of deduced amino acid sequences of sweetpoatao Ran proteins with other known Ran proteins. (A) Multiple alignment of Ran peptides from *Arabidopsis* (AtRan 1-3; At5g200010, At5g200020, At5g55190), pea (PsRan; ABM73376), yeast (ScRan; P32835), *Cenorhabditis elegans* (ran-1;.NP_499369), and human (HsRan; P62826). Lines above sequences mark the conserved region involved in GTP binding/hydrolysis motifs (G1- G4). Both the effector-binding domain (RanGAP-binding) involved in protein-protein interaction with RanGAP and the acidic C-terminal region are boxed. Identical amino acid residues are indicated by a square dot and histidine residues are indicated by asterisks. (B). Phylogenetic tree of the Ran family proteins using the DNAStar MegAlign program and a Clustal W.

resulted in the isolation of a 2,521 bp genomic fragment encoding the *IbRan2* protein. This genomic fragment has 8 exons and 7 introns, and encodes a polypeptide of 221 amino acids. The genomic sequences showed 100% identity with the corresponding region of the partial *IbRan2* cDNA, suggesting that the 2,521 bp genomic fragment (JX446680) is the gene encoding *IbRan2* cDNA. There was 95% identity between the proteins encoded in *IbRan1* and *IbRan2*.

Characterization and structure analysis of two *IbRan* genes

The alignment of amino acid sequences of Ran genes isolated from sweet potato and various other species is shown in Figure 1A. *IbRan1* and *IbRan2* showed 94 % and 97 % identity with *Arabidopsis* AtRan3 at the amino acid level. As shown in Figure 1B, *IbRan1* was more closely related to AtRan3 than to *IbRan2*. *IbRan1* also showed homology with tomato Ran1 (95%) and Ran2 (95%), wheat TaRAN1 (95%), *S. cereviciae* ScRan (78%), and human Ran/TC4 (78%). The open reading frame (ORF) region of *IbRan1* cDNA showed 81-82% identity with AtRan1-3 at the nucleotide level.

IbRan1 and *IbRan2* proteins were found to contain an effector-binding motif (KKYEPTIGVEVH) in an N-terminal. This motif is known to interact with the GTP-activating domain and shows 100% identity among known plant Ran proteins. However, two amino acids in this motif of plant Ran proteins differed from those of *Saccharomyces cerevisiae*, *Caenorhabditis elegans*, and mammalian Ran. The C-terminal is conserved in all known Ran proteins and has an acidic domain for GTP binding and protein-protein interaction. This region is an alpha-helix structure and has been found to interact with RanGAP (GTPase activating protein) and other proteins, such as RanBP1 or RCC1 in animals and plants (Ren et al.1995; Richard et al. 1995). In plant Ran, there is a stretch of five consecutive Asp residues that exist only in plants. With the exception of these five Asp residues, the C-terminal region of *IbRan2* was different from that of *IbRan1*, suggesting that they may interact with different proteins. All the aligned Ran proteins have conserved four GTP-binding sites (G1-G4), and they have histidine residues in 33 and 142 positions, which is a unique characteristic that distinguishes them from other Ras superfamily members.

Genomic southern analysis and spatial expression of two *IbRan* genes

Southern blot was carried out by using the full-length *IbRan1* cDNA as a probe. This probe detected all members of the Ran gene family due to high sequence homology and the washing conditions of the Southern blot analysis. Restriction enzymes, *Eco*R I and *Hind* III, have no restriction sites within the *IbRan1* cDNA sequences. The presence of two or three major bands and several minor ones on Southern blots indicated that *IbRan* is a multigenic family (Fig. 2).

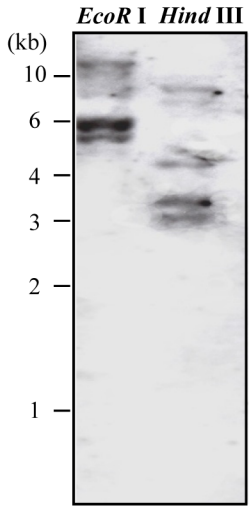

Fig. 2 Southern blot analysis of *IbRan1* gene. Genomic DNA purified from sweet potato was digested with *Eco*RI and *Hind*III, and then separated in a 0.8 % agarose gel. Southern blot analysis was carried out using the full-length *IbRan1* cDNA as a probe.

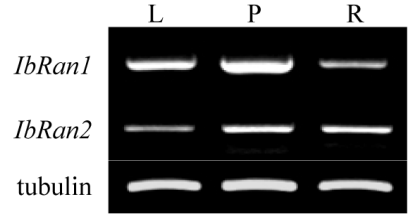

Fig. 3 Tissue-specific expression of *IbRan1* and *IbRan2* in sweet potato. Total RNA was isolated from leaves (L), petioles (P), and roots (R) of sweet potato. cDNA was synthesized from total RNA, and the PCR analysis was performed with each gene specific primers. Tubulin was used as an internal control.

We previously reported that the *IbRan1* gene is strongly expressed in suspension-cultured cells throughout the early log phase to the late log phase (Kim et al. 2006). Here we investigated the expression of *IbRan1* and *IbRan2* gene in leaf, petiole, and root tissues of sweet potato plants by reverse transcriptase (RT)-PCR analysis (Fig. 3). Specific primers were designed in the 5'-UTR and 3'-UTR of each of the two *IbRan* genes. The transcript level of *IbRan1* gene was high in petioles, moderate in leaves, and low in roots. The transcript level of *IbRan2* gene was higher in

petioles and roots than in leaves. This result was verified by the comparative analysis of three EST libraries.

Different transcriptional regulation of the two *IbRan* genes in response to various abiotic stresses

To determine whether the transcriptional regulation of these two *IbRan* genes is affected by wounding stress, we followed changes in the mRNA levels of these genes in wounded leaves, petioles, and roots. The wounding treatment consisted of cutting the leaves, petioles, and roots of sweet potato plants into small pieces and incubating these in a growth chamber for 1, 4, 8, 12 and 24 h (Fig. 4A). Control tissues were sampled prior to incubation. In leaves, *IbRan1* mRNA levels initially fell markedly, reaching a barely detectable level at 4 h after initial of the wounding treatment, followed by a dramatic increase in transcript level that eventually was much higher than the initial (baseline) level. *IbRan2* mRNA levels increased from 4 h after treatment initiation and maintained an increased expression levels up to the end of the experiment (24 h). In petioles, the *IbRan1* transcript level showed a similar pattern to that in leaves, although the final level was lower than the initial level, while *IbRan2* expression was slightly decreased at 1 h and showed no further significant change up to 24 h after treatment initiation. In roots, two *IbRan* genes exhibited a distinct transcriptional response to wounding. *IbRan1* transcript level rapidly decreased at 1 h and reached a barely detectable level at 24 h after treatment initiation. The mRNA levels of *IbRan2* showed a fluctuating pattern. The *IbRan2* transcript level was decreased at 1 h, and followed by an increase to its initial level at 8 h after wound treatment. This was followed by a second decrease in transcription levels, which showed no recovery. These results show that wounding affected *IbRan1*and *IbRan2* expression in leaves and roots, but did not affect the expression of *IbRan2* in petioles

Cold is one of the main factors affecting sweet potato growth and development. In an attempt to understand whether the *IbRan* genes are chilling-responsive genes, we analyzed the time-course accumulations of the *IbRan1* and *IbRan2* genes in three different tissue-types (leaf, petiole, and fibrous root) exposed to a non-freezing low temperature (15℃) (Fig. 4B). In leaves, the transcript levels of both *IbRan1* and *IbRan2* gradually increased during the 72 h cold treatment, while in petioles there was no significant change in the transcription levels of both genes. In roots, *IbRan1* expression showed an initial transient decrease that was followed by a gradually increase up to 72 h, whereas *IbRan2* mRNA level was slightly decreased after 48 h. This result indicates that transcription machinery of these two genes did respond differently to chilling stress in roots, but that their expression pattern is similar in petioles and leaves.

To investigate the transcriptional regulation of the two *IbRan* genes under oxidative stress, we removed two or three leaves from the top of the growing tip of sweet potato plants and incubated these in a 440 mM hydrogen peroxide solution. The *IbRan1* and *IbRan2* transcripts in leaves and petioles were then analyzed by RT-PCR. The

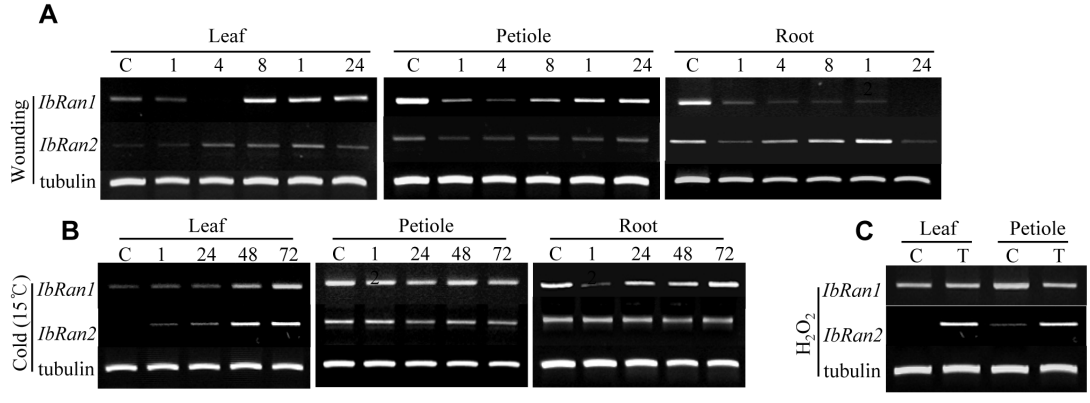

Fig. 4 Tissue-type transcriptional regulation of two *IbRan* genes in response to various stresses. Three-week-old sweet potato plants were exposed to wounding, cold, and hydrogen peroxide treatments. Total RNA was isolated from the leaves, petioles, and roots of sweetpotato. cDNA was synthesized from total RNA and PCR analysis was performed with each gene-specific primer. (A) Time-course of transcript accumulation of *two IbRan* genes following wounding stress. The wounding treatment consisted of cutting sweetpotao leaves, petioles, and roots into small pieces and incubating these at 25℃. (B) Time-course of transcript accumulation of *two IbRan* genes following exposure to cold stress. The cold treatment consisted of exposing whole sweetpoato plants to 15℃. Tubulin was used as an internal control. C, Control. (C) *IbRan1* and *IbRan2* expression following exposure to oxidative stress: sweetpotato leaves and petioles were directly exposed to 440 mM H_2O_2 at 25℃ for 24 h. C, Control. T, Treatment.

results show that hydrogen peroxide treatment did not significantly change the *IbRan1* gene transcript level in either leaves or petioles, while the expression of the *IbRan2* gene markedly increased in both tissues (Fig. 4C). This implicates that the two *IbRan* genes respond differently in leaves and petioles. Taken together, our findings suggest that the expression of these *two IbRan* genes is differentially regulated and that their regulation in response to hydrogen peroxide, wounding and cold treatments is tissue dependent.

Discussion

Ran is a key regulator of nucleoplasmic transport and cell cycle progression as well as nuclear envelope assembly. The Human and *C. elegans* genomes each contain a single Ran gene. Consequently, the multiple function of the single Ran gene is accomplished in part by the action of interacting proteins, such as RCC1, RanBP, and RanGAP. Although the high sequence homology and functional complementation suggests that plant Ran proteins may have similar roles as their mammalian and yeast counterparts, some features do differ between animal and plant Ran proteins. As yet, however, the function and regulation mechanism of each Ran isoform in plants remain to be elucidated.

Comparative analysis of the three EST libraries from sweet potato clearly showed the tissue specificity of the *Ran* (Table 1) and *RanBP* (data not shown) genes. Although the ESTs in the S-library showed a very low level of redundancy (26.4%) compared with those in the R- and L-library (84.9% and 53.8%) (Kim et al. 2006), we identified three Ran ESTs in the S-library, and one Ran EST each in the R- and L-libraries. In addition, *IbRan1* was identified as an EST specific to the exponential stage of cultured suspension cells (S-library) and photosynthetic leaf tissues (L-library), and *IbRan2* as an EST specific to early storage roots (R-library). In plants, Ran and RanBPs together with nucleoporins are considered to be involved in the regulation of various physiological responses. When we analyzed the expression pattern of *RanBP* genes in the three EST libraries, four ESTs encoding various IbRanBP isoforms were identified in the R-library, but not in the L-library, and one EST was identified in the S-library (data not shown). These data suggest that Ran protein interacting with the RanBP isoform will differ depending on cell type and/or organ within a particular plant system.

At the amino acid level, *IbRan1* and *IbRan2* were found to have 94 and 97% identity with *Arabidopsis* AtRan3, and 77 and 78% identity with human Ran/TC4, respectively. They had conserved domains, similar to those of known animal and plant Ran proteins. The highly conversed RanGAP effector-binding motif (KKYEPTIGVEV) and GTP-binding sites (G1-G4) are present in *IbRan* proteins. As shown in Figure 1A, the N- and C-terminal sequences of plant Ran protein are different from those of human, *C.elegans* and yeast. However, the N-terminal sequence is highly conserved among plant Ran proteins. *IbRan1* and *IbRan2* both contain stretch of five Asp residues in the acidic domain, which is a unique characteristic of plant Ran proteins. However, with the exception of these five Asp residues, these two Ran proteins contain different amino acid sequences in the C-terminal**.** In comparison, AtRan1 and AtRan3 show high homology except for the acidic domain, but their cellular localization and response to light signaling are different. Ren et al (1995) reported that the acidic domain of human Ran is required for the interaction between Ran and RanBP. Based on EST analysis, tissue-specificity, and C-terminal sequence, *IbRan1* and *IbRan2* will interact with the different IbRanBP isoforms. Our comparison of the 3'-UTR of the two *IbRan* genes showed that there was no significant similarity between the *IbRan1* and *IbRan2* genes.

Hydrogen peroxide treatment induces the rapid degradation of Ran protein by caspases and proteasomes in HeLa cells (Kodiha et al. 2004). Zhao et al. (2011) recently reported that shrimp Ran protein is bound to its promoter, which leads to decreased Ran promoter activity, indicating feedback regulation of *Ran* gene transcription. When we tested for progressive changes in the mRNA level of these two *IbRan* genes in three different tissue-types following exposure to wounding stress, two *IbRan* genes showed differential regulation in wounding-stressed leaves, petioles, and roots, respectively, and also showed tissue type- dependent regulation. Based on the expression pattern and relative transcript amount, *IbRan1* gene may be involved in response to wounding stress mainly in leaves and petioles, and *IbRan2* gene mainly in roots. Hydrogen peroxide treatment induced the expression of *IbRan2* mRNA in leaves and petioles, but the same treatment had no effect on *IbRan1* gene expression, suggesting that plant Ran genes may function in at least two pathways: the hydrogen peroxide-dependent signaling pathway, such as that found in mammalian cells, and an independent signaling pathway. Given the results of wounding and hydrogen peroxide treatments, we suggest that the transcripts of *IbRan1* and *IbRan2* genes are differentially regulated in a tissue-type dependent manner. Both the *IbRanI* and *IbRan*2 genes were responsible for the signaling transduction of chilling stress and that there may be functional redundancy in Ran family members against chilling stress. Plants

express three to four Ran isoforms, with the expression of each *Ran* genes being negatively or positively regulated under various environmental stresses. As such, the combination of various Ran and RanBP isoforms may target different components of the transport apparatus in plants. Further investigation of transcriptional regulation of each Ran gene and the function of each Ran isoform under different abiotic stresses will be necessary to understand the various functions of plant Ran proteins.

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