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Identification of alternatively spliced *MsRan* transcripts involved in low temperature response in *Musa* spp.

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Abstract

Ran is involved in response to external stimuli. In this study, six *MsRan* gene cDNA sequences were isolated from wild banana (*Musa* spp. AB group) from Sanming City, China. Sequence analysis reveals that MsRan3A, MsRan3A-1a, and MsRan3C contained Ran protein domains including a GTP hydrolysis domain, a RanGAP-binding domain, and an acidic tail, whereas two G boxes (G4 and G5) were absent in MsRan3A-6a. The physicochemical property of MsRan3A, MsRan3A-1a, MsRan3A-6a, and MsRan3C appeared to differ significantly. Real time quantitative PCR (qPCR) analysis indicates that *MsRan3A-1*, *MsRan3A-5*, *MsRan3A-6*, *MsRan3A-6a*, and *MsRan3C-1* were expressed in roots, leaves, peduncles, bracts, flowers, peels, and pulp of the wild banana. *MsRan3A-1a* was expressed at extremely low levels in these tissues and was undetectable by qPCR. The *MsRan* genes were found to be involved in responses to a low temperature stress but with different response patterns. Furthermore, salicylic acid significantly enhanced *MsRan* gene expressions suggesting the involvement of these genes in salicylic acid signal transduction.

Additional keywords: gene expression, salicylic acid, wild banana.

Introduction

Banana (*Musa* spp.), an important food staple and income-generating fruit crop in tropical and subtropical regions, is sensitive to biotic and abiotic stresses such as pests, drought, and low temperatures. In particular, a low temperature has a serious impact on banana yield and fruit quality (Yang *et al.* 2012). In a previous study, we identified several wild banana populations with cold resistance, including one from Sanming, Fujian Province, China (Lai *et al.* 2006, 2007). The identification of cold resistance related genes from cold resistant wild banana and the investigation of the underlying mechanisms are useful for genetic improvement and production of banana.

Ran, a Ras-related nuclear protein, belongs to a highly conserved small G protein family present in eukaryotic organisms. In mammals, Ran is involved in multiple cellular processes (Ciciarello *et al.* 2007, Clarke and Zhang 2008, Dallol *et al.* 2009). It also participates in cellular signal transduction (Liu *et al.* 2010). *Arabidopsis* contains four *Ran* genes (Vernoud *et al.* 2003). Genomic analysis revealed the presence of nine *Ran* genes in the genome of *Musa acuminata* (D'Hont *et al.* 2012, Zhang *et al.* 2015b). Studies have revealed that plant Ran proteins share a high sequence similarity with their counterparts from yeast and animals and function similarly (Ach and Gruissem 1994, Wang *et al.* 2004, 2006, Yano *et al.* 2006, Lee *et al.* 2008, Zang *et al.* 2010, Lü *et al.* 2011). Plant Ran proteins are expressed in various tissues, especially in meristematic tissue (Ach and Gruissem 1994, Haizel *et al.* 1997, Wang *et al*. 2006, Lü *et al*. 2011). Zang *et al*. (2010) have demonstrated that a reduced expression of *OsRan2* causes developmental

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Abbreviations: AUAP - abridged universal amplification primer; BA - 6-benzyladenine; MS - Murashige and Skoog; NAA - naphthalene acetic acid; ORF - open reading frame; pI-isoelectric point; qPCR - quantitative PCR; RACE - rapid amplification of cDNA ends; SA - salicylic acid; UTR - untranslated region.
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abnormalities in rice, and Chen *et al.* (2011) have shown that development in *OsRAN2*-knockdown rice plants is delayed, and spindle organization is aberrant. These results indicate that Ran has a fundamental role in plant development.

According to several studies, Ran is also involved in responses to an external stimulus. For example, Ran protein expression is enhanced under heat stress (Ferreira *et al.* 2006, Xu and Huang 2008, 2010), and low temperatures stimulate *Ran* transcript accumulation as well (Chen *et al*. 2011, Paul and Kumar 2011). Miché *et al.* (2006) revealed that jasmonic acid, a plant hormone that participates in signaling multiple stresses, induces Ran protein expression in rice roots. Lee *et al*. (2008) determined that various light sources regulate expression of the *Ran* gene through phytochrome-mediated signaling pathways. An enhanced accumulation of the Ran protein has also been found in NaCl-treated *Arabidopsis* roots (Jiang *et al.* 2007). Two Ran GTPases were found to accumulate in roots of wild watermelon under water deficits. Lee *et al*. (2008) reported that various light sources regulate *Ran* gene expression through phytochrome-mediated signaling pathways. Wang *et al*. (2006) demonstrated that overexpression of *TaRAN1* renders *Arabidopsis* hypersensitive to auxin. Zang *et al*. (2010) has reported similar results in rice with transgenic plants overexpressing *OsRAN2* exhibiting an enhanced sensitivity to salinity, osmotic stress, and abscisic acid. Chen *et al*. (2011) further indicated that *OsRAN2*-overexpressing transgenic rice displays an enhanced cold tolerance. These authors suggested that *OsRAN2* maintains cell division by promoting the normal export of intranuclear tubulin at the end of mitosis and regulates cold resistance in rice. The results of these studies imply that *Ran* genes play a crucial role in plant stress response.

Fifteen DNA sequences and six cDNA sequences of *Ran* genes have been isolated from wild banana previously (Zhang *et al.* 2014, 2015a). In this study, we isolated *MsRan* genes and alternative spliced transcripts from the wild banana. We then assessed their tissue-specific expression profiles as well as their expressions under low temperature stresses and salicylic acid (SA) treatment.

Materials and methods

Plants and treatments: Wild banana (*Musa* spp. AB group) from Sanming city was grown in our germplasm repository (the Fujian Agriculture and Forestry University, Fuzhou, Fujian Province, China). Suckers were collected, rinsed, and treated as described by Zhang *et al.* (2006). The explants were inoculated into a half-strength Murashige and Skoog (MS) medium (pH 5.8) supplemented with 30 g dm^{-3} sucrose, 6.0 g dm^{-3} agar, 3.0 mg dm^{-3} 6-benzyladenine (BA), 0.1 mg dm^{-3} naphthaleneacetic acid (NAA), and 0.5 g dm^{-3} active carbon and cultivated at 28 ± 1 ºC under the dark. After budding, the cultures were transferred and maintained at a temperature of 28 ± 1 °C, a 12-h photoperiod, and an irradiance of 200 μmol m^{-2} s^{-1}. For propagation, the established cultures were transferred to an MS medium supplemented with 30 g dm^{-3} sucrose, 6.0 g dm^{-3} agar, 1.0 mg dm^{-3} 6-BA, and 0.1 mg dm^{-3} NAA (pH 5.8). For cold and SA treatments, shoot cultures subcultured on the propagation medium for 20 d were used. For tissue-specific expression analysis, we collected roots, leaves, peduncles, bracts, flowers, peels, and pulp from adult plants with fruits at 60 % maturity (Fig. 1 Suppl.). To examine the responses of wild banana to low temperature stresses, shoot cultures were exposed to various temperatures (0, 4, 8, 13, 20, and 28 °C) for 36 h and to 8 °C for various time periods (0, 1, 4, 8, 12, and 24 h). Salicylic acid treatment was carried out by spraying shoot cultures with distilled water containing 0 (control) or 0.5 mM SA. Both control and SA-treated shoot cultures were subsequently incubated under 8 °C. Samples were taken at 1, 4, 8, 12, and 24 h. The tissues and treated shoot cultures were harvested and immediately frozen in liquid nitrogen and stored at -80 °C until further use.

Cloning *MsRan* genes: The total RNA was extracted from the frozen samples using an *EZNA* plant RNA kit (*Omega Bio-Tek,* Norcross, GA, USA) followed by treatment with DNase I (*Takara Biotechnology*, Dalian, China) to remove genomic DNA. The cDNA was synthesized with a *RevertAid*™ first-strand cDNA synthesis kit (*Fermentas, Thermo Fisher Scientific*, Waltham, MA, USA). *MsRan* gene fragments were amplified using three primer pairs (RanF-4/RanR-4, RanF-5/RanR-5, and RanF-8/RanR-8) designed according to *Ran* gene sequences from the *Musa acuminata* genome (http://banana-genome.cirad.fr/) and from the National Center for Biotechnology Information (*NCBI*) database. To clone the full-length cDNA sequence of *MsRan* genes, we carried out a rapid amplification of cDNA ends (RACE) using primers designed according to the obtained *MsRan* gene fragments. The 3′-RACE was performed using a first-strand cDNA synthesis kit (*Fermentas*) with an oligo-dT adapter primer (AP). The cDNA was then subjected to nested PCR using gene-specific primers (Ran3′RACE-1, Ran3′RACE-2, and Ran3′RACE-3) and a universal amplification primer (AUAP). In the case of 5′-RACE, the first-strand cDNA was synthesized using an oligodT primer and *SuperScript*™ *III* (*Invitrogen,* Carlsbad, CA, USA), and the product was purified using a *MiniBEST* DNA fragment purification kit (*Takara*). A homo-polymeric tail was then added to the cDNA 3′-end using

terminal deoxynucleotidyl transferase (*Takara*) and dCTP. Nested PCR was carried out with two upstream primers (AP3 and AUAP) and three gene-specific downstream primers (Ran5′RACE-1, Ran5′RACE-2, and Ran5′RACE-3) to amplify the 5′-end of *MsRan* genes. The full-length *MsRan* was amplified by RT-PCR using primers designed according to 5′- and 3′-untranslated region (UTR) sequences. After resolving the PCR products on a 1 % (m/v) agarose gel, the bands were excised, purified, subcloned into a pMD18-T vector (*Takara*), and sequenced. All primers used for isolating *MsRan* genes are listed in Table 1 Suppl.

Bioinformatics analysis: Nucleotide and deduced amino acid sequences and amino acid sequence alignments were analyzed by the *DNAMAN* software. The open reading frames (ORFs) of *MsRan* cDNAs were determined using *ORF Finder* (www.ncbi.nlm.nih.gov/gorf/) and translated into the corresponding amino acid sequence. Homology comparisons were conducted using the *BLAST* program at the *NCBI* (http://www.ncbi.nlm.nih.gov/blast). The theoretical isoelectric point (pI) and mass values of mature peptides were calculated using the *ProtParam* tool (http://web.expasy.org/protparam). A phylogenetic tree was constructed by the neighbor-joining method with 1 000 bootstrap replicates in *MEGA 5*.

Real-time quantitative PCR: The total RNA (500 ng) was transcribed into cDNA with random primers and an oligodT primer using a *PrimeScript RT* reagent kit (*Takara*). Real time quatitative PCRs (qPCRs) were performed on a *Lightcycler 480* system (*Roche Applied Science*, Basel, Switzerland) as described by Fang *et al.* (2016). Reactions were run in three biological replicates and three technical replicates using 18S rRNA as an internal control. Analyses of expression data were performed with *GeNorm v. 3.5* (Vandesompele *et al.* 2002). The specificity of the products from the real time qPCR experiments were confirmed by melting curve analysis and sequencing. All primers are listed in Table 2 Suppl.

Results

Six *MsRan* cDNAs were isolated from wild banana by real time PCR coupled with 5'- and 3'-RACE using primers listed in Table 1 Suppl. Sequence analysis indicates that *MsRan3A-1*, *MsRan3A-5*, *MsRan3A-6*, and *MsRan3C-1* contained a 663-bp ORF, whereas *MsRan3A-1a* and *MsRan3A-6a* contained ORFs of 585 and 483 bp, respectively. The comparison of nucleotide sequences demonstrates that they were highly homologous. The sequences of *MsRan3A-1* and *MsRan3A-1a* were identical and the sequences of *MsRan3A-5*, *MsRan3A-6*, and *MsRan3A-6a* were similar to one another. The genomic sequence encoding *MsRan3A-1* and *MsRan3A-1a* (GenBank accession No. KC898288) and the ones encoding *MsRan3A-5*, *MsRan3A-6*, and *MsRan3A-6a* (GenBank acc. No. KC898291) have been previously isolated (Zhang *et al.* 2014). The comparative structural analysis of *MsRan3A-1* and *MsRan3A-1a* reveal that the coding region of *MsRan3A-1a* lacked a 78-bp exon (Fig. 1). The comparison of the structures of *MsRan3A-5*, *MsRan3A-6*, and *MsRan3A-6a* indicates that the coding region of *MsRan3A-6a* lacked a 180-bp exon and that the 3′-UTR of *MsRan3A-5* lacked a 75-bp sequence (Fig. 1).

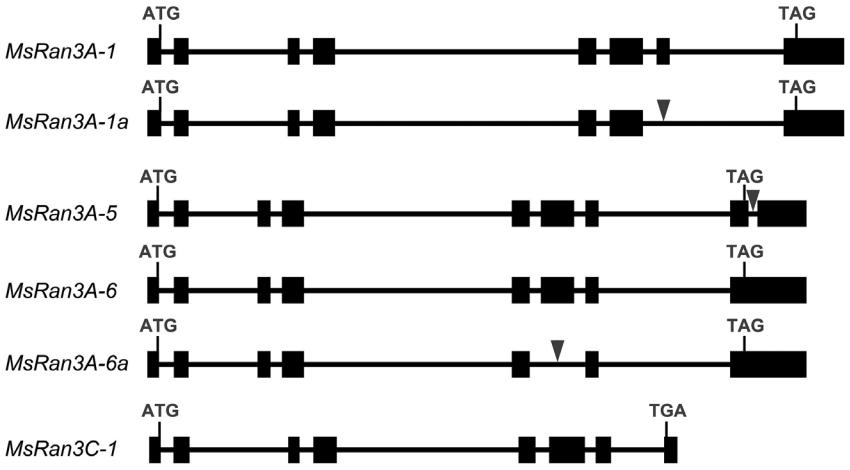

Fig. 1. Schematic representations indicate splicing patterns of *MsRan* gene transcripts. *Bold lines* indicate introns, *black boxes* indicate exons, ATGs indicate start codons, TAGs indicate termination codons, *arrows* indicate locations where sequence deletions exist.

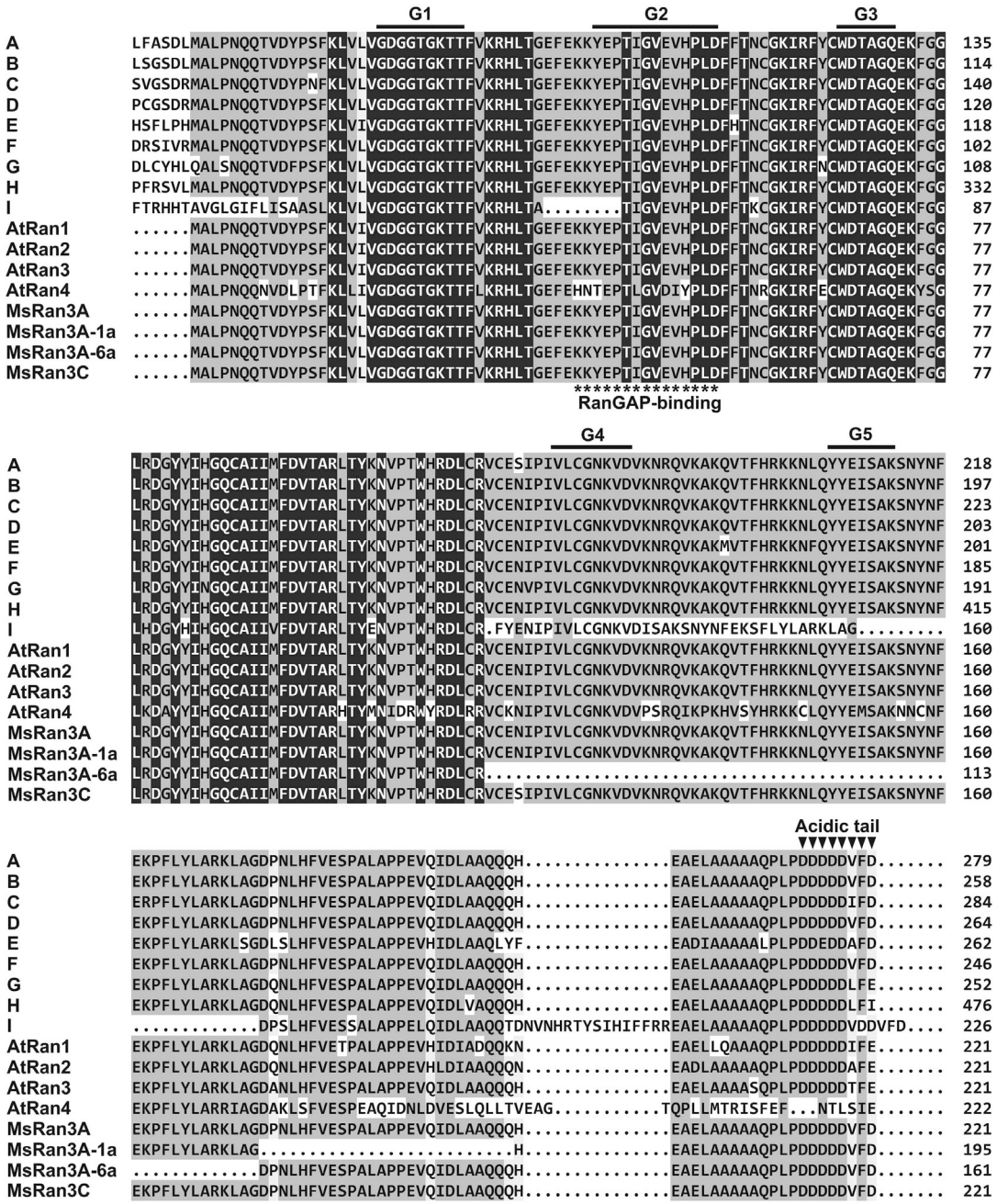

Fig. 2. Multiple alignments of Ran sequences from banana and *Arabidopsis*. *A*-*H* - predicted Ran proteins from the banana genome encoded by *A*: Achr1T05300, *B*: Achr1T11790, *C*: Achr1T14580, *D*: Achr4T00780, *E*: Achr5T06130, *F*: Achr5T09850, *G*: Achr6T01500, *H*: AchrUn_randomT07030, *I*: GSMUA AchrUn randomP07030. Conserved GTP binding and hydrolysis domains (G1-G5) are indicated by *bold lines*. An effector-binding domain (RanGAP-binding) and an acidic C-terminal region (acidic tail) are indicated with *asterisks* and *triangles*, respectively.

MsRan3A-1, *MsRan3A-5*, and *MsRan3A-6* were found to encode a protein of 221 amino acids with a predicted molecular mass of 25.1 kDa and a pI of 6.38. A protein encoded by *MsRan3C-1* was also found to comprise 221 amino acids with a predicted molecular mass of 25.0 kDa and a pI of 6.38. Sequence deletions in *MsRan3A-1a* and *MsRan3A-6a* were responsible for the absence of 26 and 60 amino acids in their deduced proteins, respectively. A predicted molecular mass and pI of MsRan3A-1a were 22.3 kDa and 8.15, respectively, whereas corresponding values in MsRan3A-6a were 18.1 kDa and 4.88. Analysis by *BLAST* reveals that predicted MsRan proteins shared a high similarity with Ran homologs from other plants. Multiple alignments with Ran homologs from *Arabidopsis* and *M. acuminata* indicate that MsRan3A, MsRan3A-1a, and MsRan3C

contained several conserved motifs including G boxes (G1 - G5), a RanGAP-binding domain and an acidic C-terminal region (Fig. 2). In MsRan3A-6a, however, G4 and G5 domains, known to be involved in GTP-binding and hydrolysis, were lacking. To study the relationships of predicted MsRan proteins and their orthologs in other species, we generated a phylogenetic tree of deduced amino acid sequences of the MsRans and 19 other Ran homologs. In the phylogenetic tree, the Ran sequences from banana, except for MsRan3A-1a and Achr5T06130, fell into the same clade and were closely related to AtRan3. This placement suggests that MsRan3A, MsRan3A-6a, MsRan3B, MsRan3C, and MsRan3D share a conserved role with Ran3 (Fig. 3).

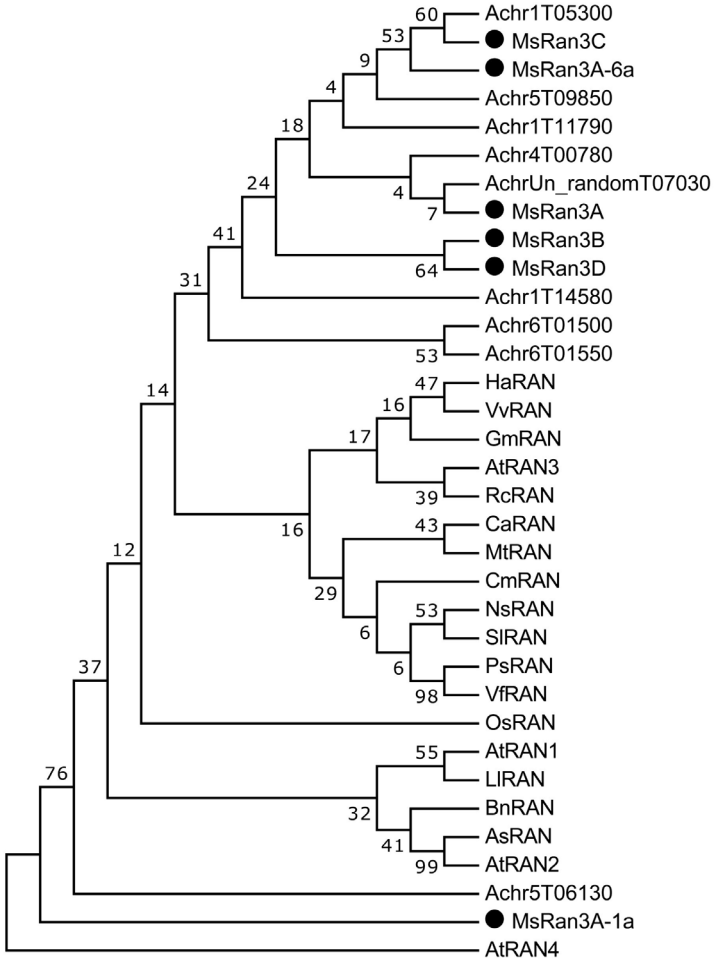

Fig. 3. A phylogenetic tree of deduced amino acid sequences of Ran proteins from *Musa acuminata* and other plant species. Phylogenetic and evolutionary analyses were performed using the neighbor-joining method by the *MEGA 5* software. Additional sequences include Ran proteins from *Allium sativum* (AsRAN, ABD17865), *Arabidopsis thaliana* (AtRAN1, NP_197501, AtRAN2, NP_197502, AtRAN3, NP_200330), *Brassica napus* (BnRAN, ABD17866), *Cicer arietinum* (CaRan, NP_001265929), *Cucurbita maxima* (CmRAN, AEK84227), *Glycine max* (GmRan, XP_003522628), *Helianthus annuus* (HaRan, AF495716_1), *Lepidium latifolium* (LlRAN, AEK78856), *Medicago truncatula* (MtRAN, ACJ83982), *Musa acuminata* (as described in Fig. 2), *Nicotiana sylvestris* (NsRan, NP_001289503), *Oryza sativa* (OsRAN, NP_001043550), *Pisum sativum* (PsRAN, ABM73376), *Ricinus communis* (RcRan, XP_002515555), *Solanum lycopersicum* (SlRAN, NP_001234016), *Vicia faba* (VfRAN, P38548), and *Vitis vinifera* (VvRAN, XP_002284967).

Real time qPCR analysis was performed to assess the expression pattern of the *MsRan* genes in different wild banana tissues (Fig. 4). *MsRan3A-1*, *MsRan3A-5*, *MsRan3A-6*, *MsRan3A-6a*, and *MsRan3C-1* were expressed in all tested tissues. *MsRan3A-1*, *MsRan3A-5*, and *MsRan3C-1* were weakly expressed in roots, peduncles, bracts, and peels with the lowest expression in roots, and were highly expressed in leaves, flowers, and pulp. *MsRan3A-6* and *MsRan3A-6a* had the highest expressions in leaves followed by peduncles and showed the lowest expression in bracts. Interestingly, only a small amount of *MsRan3A-1a* was detected in any tested tissue (Ct values were greater than 34).

To investigate the effects of low temperatures on *MsRan* gene expressions, we subjected *in vitro* seedlings of wild banana to various temperatures (Fig. 5).

Expression of *MsRan3A-1* decreased slightly under 20 °C treatment and increased to a higher level under 13 °C treatment. When exposed to a temperature of 8 °C, *MsRan3A-1* expression was reduced to the lowest level, at even lower temperatures (4 and 0 °C), however, its expression was enhanced. As temperatures were lowered from 28 to 13 °C, expression of *MsRan3C-1* increased. Similar to *MsRan3A-1*, *MsRan3C-1* showed the lowest expression under 8 °C with even lower temperatures enhancing its expression (Fig. 5). The lowest expressions of *MsRan3A-5*, *MsRan3A-6*, and *MsRan3A-6a* were detected during 20 °C treatment. Expressions of these genes gradually increased as temperature was lowered from 20 to 8 °C, and the highest expressions were detected at 0 °C.

We also performed time-course experiments involving 8 °C treatment. Expressions of *MsRan3A-1*, *MsRan3A-5*, *MsRan3A-6*, and *MsRan3A-6a* increased slightly from 0 to 4 h, and after 8 h of treatment, their expressions rapidly decreased to their lowest levels and remained there (Fig. 6). Conversely, expression of *MsRan3C-1* was reduced after 1 h of treatment and then rose after 4 h to pre-treatment levels. Similar to other transcripts, *MsRan3C-1* was expressed at the lowest level after 8 h of treatment and remained at a low level thereafter (Fig. 6). These data suggest that *MsRan* genes are involved in a response to low temperature stress with various transcripts responding to low temperatures according to different patterns.

We also examined the effects of SA on expressions of *MsRan* genes under low temperature stress (Fig. 7). *MsRan* gene expressions were significantly enhanced after 1, 8, 12, and 24 h of SA treatment at 8 °C. After 4 h of SA treatment, however, *MsRan3A-1*, *MsRan3A-6*, *MsRan3A-6a*, and *MsRan3C-1* expressions were only slightly repressed, whereas *MsRan3A-5* expression was unaffected. These results imply that *MsRan* genes participate in SA signalling.

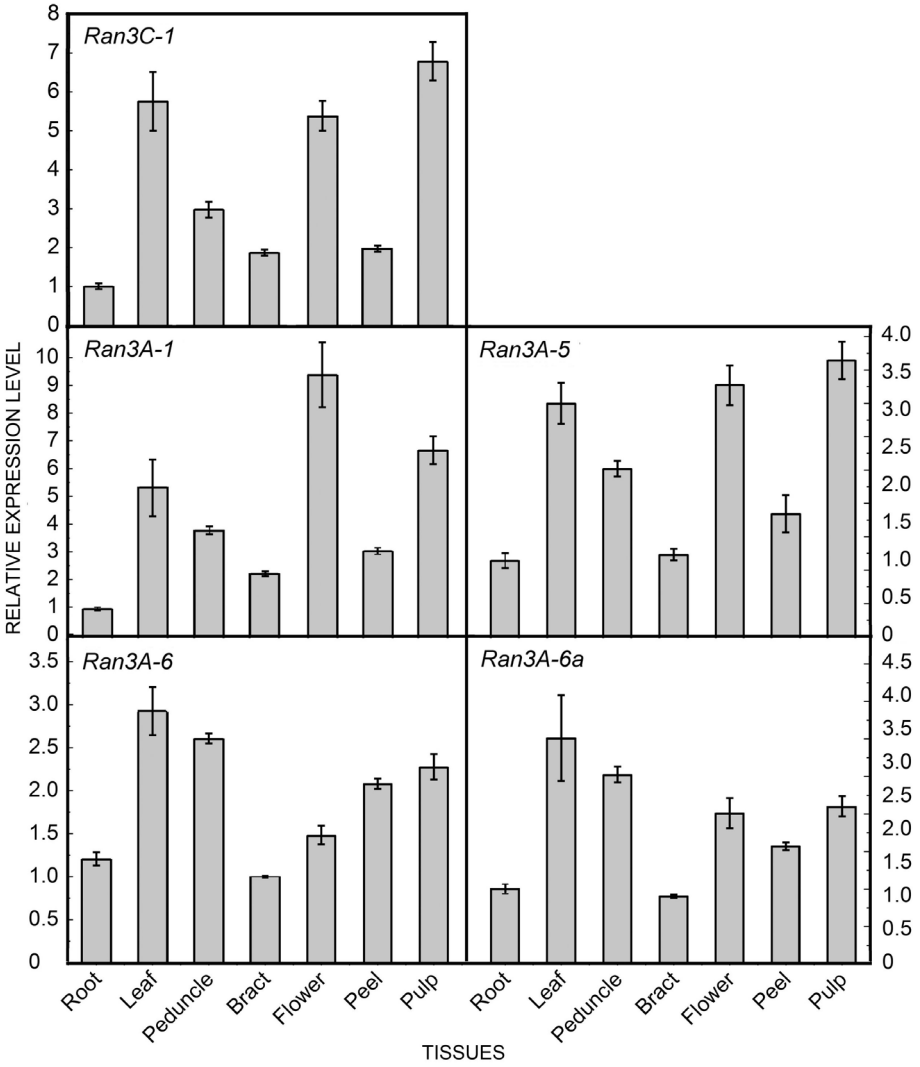

Fig. 4. Expression analysis of *MsRan* genes in various tissues of adult wild banana. The total RNA was extracted from various tissues and subjected to real time quantitative PCR analysis. All data were normalized to the expression of 18S rRNA. Means ± SEs, $n = 3$.

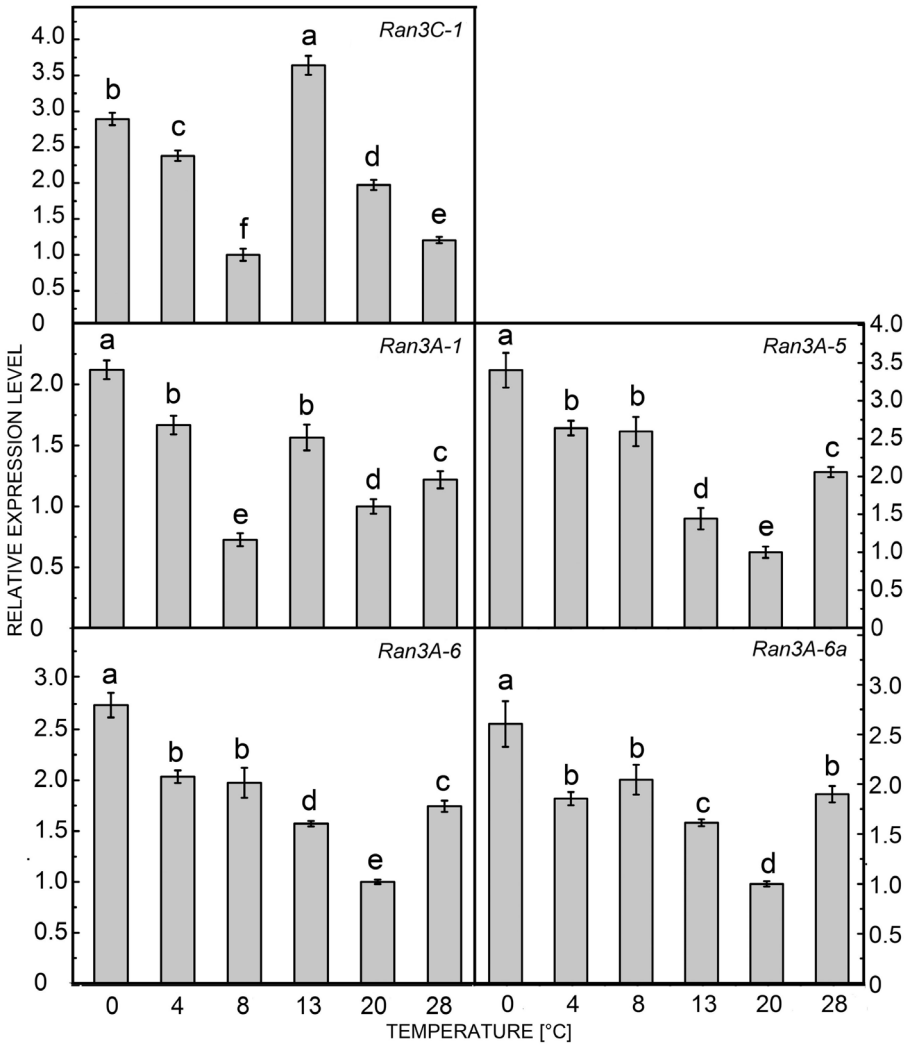

Fig. 5. Expression analysis of *MsRan* genes under low temperature stresses. The total RNA was extracted from shoot cultures treated by different temperatures and subjected to real time qPCR analysis. All data were normalized to the expression of 18S rRNA. Means ± SEs, $n = 3$. Significant differences were determined by one-way *ANOVA* ($P < 0.05$) and are indicated by different letters.

Discussion

Plant *Ran* proteins are abundantly expressed in meristematic tissue (Haizel *et al*. 1997, Lü *et al*. 2011). Fang *et al.* (2014) have demonstrated that *DlRan* transcripts are highly expressed during developmental stages when cells are actively proliferating. Wang *et al*. (2006) reported that *TaRAN1*-overexpressing *Arabidopsis* plants exhibit increased primordial meristems, tillering, apical dominance, and the number of rosette leaves and of cells in the root meristem zone. These studies suggest that Ran is also involved in regulation of cell division. Our results indicate that predicted MsRan proteins are highly similar to Ran proteins from other plants. *MsRan* transcripts were weakly expressed in old tissues and highly expressed in young ones. Furthermore, their expressions were significantly reduced after treatment at 8 °C for 8 h. It was reported that banana growth is arrested and injury occurs under this temperature (Yang *et al*. 2012). However, different transcripts showed varied expression patterns. The various *MsRan* genes may thus have divergent functions. Further investigation is needed to determine their exact function.

Alternative splicing is a post-transcriptional regulatory mechanism that plays important roles in regulation of gene expression (Lareau *et al.* 2004, Stamm *et al.* 2005). This is a powerful mechanism for regulation of plant stress response (Mastrangelo *et al.* 2012). Alternatively spliced *Ran* transcripts have rarely been identified. Fang e*t al*. (2016) isolated an alternatively spliced *DlRan* transcript containing a premature terminator codon from somatic embryos of longan and

proposed its involvement in regulation of the *DlRan* gene expression. In the present study, three alternatively spliced transcripts of *MsRan* were isolated from wild banana. Exon deletions were detected in the coding regions of *MsRan3A-1a* and *MsRan3A-6a*. The predicted protein encoded by *MsRan3A-1a* contained all of the conserved motifs, whereas that of *MsRan3A-6a* lacked the G4 and G5 boxes, which are involved in GTPase hydrolysis. *MsRan3A-1a* was not detected in any tested sample, whereas *MsRan3A-1* was differentially regulated in various tissues as well as under the low temperature and SA treatments. In contrast to *MsRan3A-6*, *MsRan3A-6a* was found to be more abundant in flowers than in peels (Fig. 3). We propose that *MsRan3A-1a* and *MsRan3A-6a* may have different functions than their wild-type counterparts. The exon deletion in *MsRan3A-5* was found in the 3′-UTR. The 3′-UTR contains various regulatory elements and plays roles in post-transcriptional regulation of gene expression (Garneau *et al.* 2007, Keene 2007). Unlike *MsRan3A-6*, the highest expression of *MsRan3A-5* was detected in flowers and pulp. These results suggest that *MsRan3A-5* and *MsRan3A-6* might function differently. Further study is needed, however, to elucidate whether the exon-deletion in the 3′-UTR of *MsRan3A-5* has altered its function.

Plants respond to stresses by reprogramming gene expression, thereby giving rise to metabolic alterations. To activate gene expression, environmental signals must be transduced into a nucleus through a series of signal transduction pathways. Nucleo-cytoplasmic partitioning of proteins is proposed as a vital mechanism involved in regulation of plant response to environmental signalling (Merkle 2003, Meier and Somers 2011). These results confirm the importance of Ran, which plays a role in nucleo-cytoplasmic trafficking proteins.

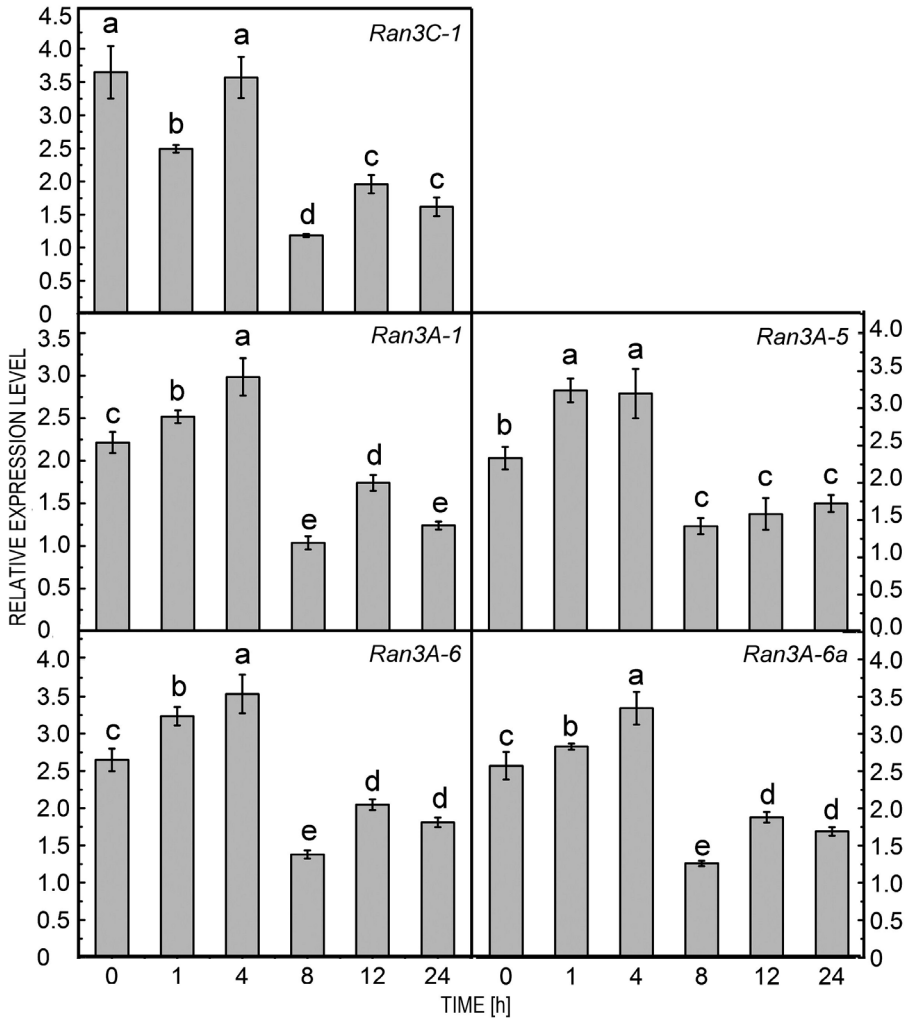

Fig. 6. The effects of a low temperature (8 °C) on expression of *MsRan* genes in wild banana. The total RNA was extracted from 8 °C treated shoot cultures at different time points and subjected to real time qPCR analysis. All data were normalized to the expression of 18S rRNA. Means ± SEs, $n = 3$. Significant differences were determined by one-way *ANOVA* ($P < 0.05$) and are indicated by different letters.

Ran was shown to participate in response to stresses in several plants (Ferreira *et al*. 2006, Jiang *et al*. 2007, Li *et al.* 2007, Xu and Huang 2008, 2010, Yoshimura *et al.* 2008, Chen *et al*. 2011, Paul and Kumar 2011). Chen *et al*. (2011) reported that 4 °C treatment of rice significantly enhances expression of *OsRAN2*. However, we found that expressions of *MsRan* genes increased slightly after 1 and 4 h, then decreased and remained at a low level after the seedlings were treated at 8 °C for 8 h. When the seedlings were exposed to different temperatures, the abundance of *MsRan* genes varied. We thus propose that the differences of temperature are responsible for the discrepancy between our results and the previous study. The low expression of *MsRan* transcripts when treated by 8 °C for more than 8 h may be related to growth arrest and injury, which may occur under this temperature (Yang *et al*. 2012). Our results also indicate that *MsRan* genes respond to low temperature in various patterns. When the temperature was lower than 8 °C, expressions of *MsRan3A-1* and *MsRan3C-1* were re-enhanced. These could be due to that the banana seedlings adapted their metabolic processes to cope with the cold stress. These results suggest that *MsRan* genes are involved in a response to low temperature stress.

Salicylic acid plays an important role in modulating plant responses to abiotic and biotic stresses (Senaratna *et al.* 2000, Ding *et al.* 2002, Tasgin *et al.* 2006, Wen *et al.* 2008, Fu *et al.* 2011, Saruhan *et al.* 2012, Mutlu *et al.* 2013b.

Salicylic acid treatment increases chilling tolerance of many plants (Mutlu *et al.* 2013a). Kang *et al*. (2003a,b)

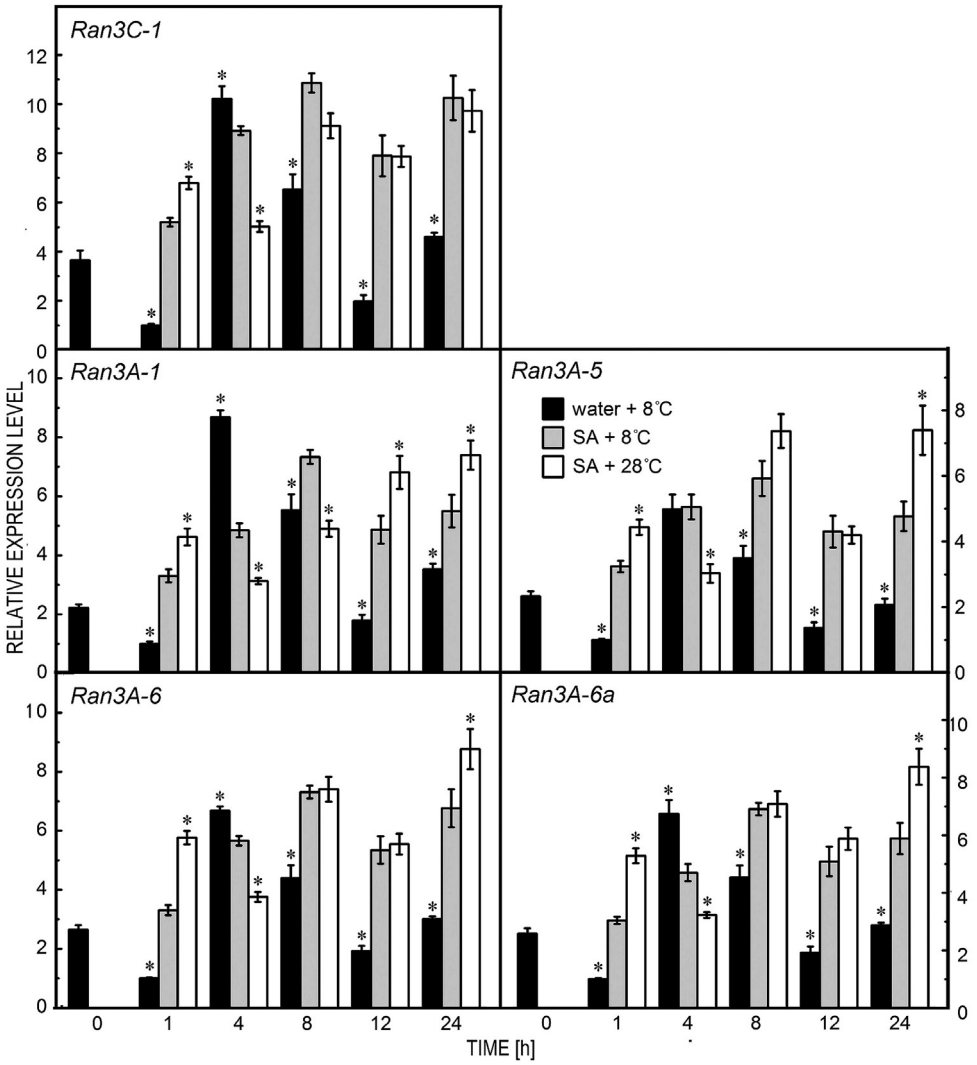

Fig. 7. Expressions of *MsRan* genes under salicylic acid (SA) treatment in wild banana. Shoot cultures sprayed with 0.5 mM SA or water (control) and incubated under 8 °C. The total RNA was extracted from treated and control plantlets at different time points and subjected to real time quantitative PCR analysis. All data were normalized to the expression of 18S rRNA. Means ± SEs, $n = 3$. Significant differences were determined by one-way *ANOVA* ($P < 0.05$) and indicated by *asterisks*.

demonstrated that SA treatment enhances chilling tolerance of banana seedlings. In the present study, *MsRan* gene expressions in seedlings treated with 0.5 mM SA were significantly enhanced. Especially at the end of the treatment, the *MsRan* genes were still expressed at a high level. We propose that SA modulates *MsRan* expression, and a high expression of *MsRan* genes induced by SA is responsible for an enhanced chilling tolerance of banana.

In conclusion, six *MsRan* transcripts including three alternatively spliced transcripts were isolated from wild banana and bioinformatically characterized. The high similarity of these sequences to other plant Ran sequences suggests the former are orthologs. The *MsRan* genes were differentially regulated in various tissues, and they were involved in responses to the low temperature stress. Also, SA significantly enhanced expression of the *MsRan* genes.

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