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Molecular cloning, characterization, and expression analysis of a gene encoding a Ran binding protein (RanBP) in *Cucumis melo* L.

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Abstract: Ran binding proteins (RanBPs) are highly conserved members of the GTP-binding protein family that are involved in nuclear protein export between the nucleus and the cytoplasm. In this study, a *CmRanBP* gene from a melon was isolated (*Cucumis melo* L.) using the RACE (rapid amplification of cDNA ends) method. The 778 basepair long melon, with a RanBP cDNA encoding consisting of 197 amino acids (22.2 kDa protein), was characterized (GenBank accession no: EU853459). The predicted amino acid sequence of *CmRanBP* was found to be 70% identical to *VvRanBP*, *PtRanBP*, and *RcRanBP* from *Vitis vinifera*, *Populus trichocarpa*, and *Ricinus communis*, respectively. Within the RanBD (Ran binding domain), 5 highly conserved motifs and 1 Ran binding motif were found in all members of the *RanBP* gene family from various plant species. Expression profiles of the *CmRanBP* gene in different tissues under high temperature stress were also investigated by semiquantitative RT-PCR. The *CmRanBP* gene was expressed in a similar manner in the roots, leaves, and stems at 25 °C as a control environment. However, when the temperature was raised to 38 °C and 40 °C, expression levels of the *CmRanBP* gene were significantly ($P < 0.05$) increased in the root, leaf, and stem tissues. We show here for the first time that the *CmRanBP* gene expression was correlated with heat stress responses.

Key words: Melon (*Cucumis melo* L.), Ran binding protein (*RanBP*), gene cloning, gene expression, heat stress

Kavunda (*Cucumis melo* L.) Ran bağlayıcı proteini (RanBP) kodlayan genin moleküler klonlanması, karakterizasyonu ve ifade analizi

Özet: GTP-bağlayıcı protein ailesinin yüksek derecede korunmuş üyesi olan Ran bağlayıcı proteinleri (RanBPs) stoplazma ve çekirdek arasındaki çekirdek proteinlerin ihracatına katılmaktadırlar. Bu çalışmada, kavundan (*Cucumis melo* L.) RACE metodu kullanılarak *CmRanBP* geni izole edilmiştir. 778 baz çifti uzunluğunda ve 197 amino asiti (22,2 kDa protein) kodlayan kavun RanBP cDNA'sı karakterize edilmiştir (GenBank erişim no: EU853459). *CmRanBP* genine ait tahmini aminoasit sekansının *Vitis vinifera*, *Populus trichocarpa* ve *Ricinus communis* bitkilerine ait sırasıyla *VvRanBP*, *PtRanBP*, and *RcRanBP* genleri ile % 70 oranında benzerlik gösterdiği bulunmuştur. Çeşitli bitki türlerinde RanBD (Ran bağlayıcı domain) içinde *RanBP* gen ailesinin bütün üyelerinde yüksek oranda korunumlu beş motif ve bir tane Ran bağlayıcı motif bulunmuştur. Yüksek sıcaklık stresi altında farklı dokularda *CmRanBP* gen ifade profilleri de yarı kantitatif RT-PCR ile incelendi. Kontrol ortamı olarak 25 °C'de *CmRanBP* geni kök, yaprak ve gövdelerde benzer bir şekilde ifade edilmiştir. Ancak, sıcaklık 38 °C ve 40 °C' ye yükseltildiği zaman, kök, yaprak ve gövde dokularında *CmRanBP* gen ifade düzeyi anlamlı ($P < 0,05$) şekilde artmıştır. Biz burada ilk kez *CmRanBP* gen ifadesinin ısı stres yanıtları ile ilişkili olduğunu göstermekteyiz.

Anahtar sözcükler: Kavun (*Cucumis melo* L.), ran bağlayıcı protein (RanBP), gen klonlama, gen ifadesi, ısı stresi

Introduction

Melon (*Cucumis melo* L.), which belongs to the family *Cucurbitaceae*, is an important horticultural crop grown in temperate, subtropical, and tropical regions worldwide. The total melon production in 2007 exceeded 930 million metric tons, 83.5% of which is produced in Asia. The remainder is cultivated in America (6.5%), in Europe (5.5%), and in Africa (4.5%) (1). Melon has the potential to become a model plant for understanding important traits in fruiting crops. Melons show a wide range of morphological, physiological, and biochemical diversity (2-4), which can be examined carefully in terms of flavor development and changes in texture that occur during fruit ripening.

Melons and other cucurbits are susceptible to heat, moisture, and ultraviolet radiation during production. Critical susceptible periods include flowering, fruit expansion, and sugar accumulation. Sunburn is a major problem after canopy collapse and when fruits are standing in the field at harvest.

Small GTP-binding proteins are monomeric G proteins, whose molecular masses range between 20 and 40 kDa. They comprise a superfamily of more than 100 small G proteins present in eukaryotes from yeast to human (5). This superfamily can be structurally classified into at least 5 families: Ras, Rho, Rab, Sar1/Arf, and Ran (6). Among this superfamily, members of the Ran family play a central role in nucleocytoplasmic transport during the G1, S, and G2 phases of the cell cycle (7-10) and in microtubule organization during the M phase (11-13).

Ran is a small, nuclear GTP-binding protein which is periodically activated and inactivated like other small G proteins. The distinctive feature of the Ran cycle is that the GTP-bound form and the GDP-bound form are found in the nucleus and the cytoplasm, respectively. They are regulated by the action of interacting proteins such as the Ran nuclear guanine nucleotide exchange factor (RCC1), the Ran-GTPase activating protein (RanGAP), the Ran binding protein (RanBP), and the guanine nucleotide release factor (Mog1) (14).

In animals, RanBPs and their regulatory factors play critical roles in controlling nuclear processes throughout the cell mitotic cycle (15). In Arabidopsis, yeast 2 hybrid and in vitro protein interaction analyses indicated that *At-RanBP1a* and *At-RanBP1b* interact with the GTP-bound form of Ran proteins (Ran1, Ran2, and Ran3) (16). This suggests that they might be functioning similar to their yeast homologs (16-18). Although the protein sequence of RanBPs from plants showed a high similarity to those from animals and yeast, the difference between animal and plant Ran proteins may arise from their regulatory factors, such as RanBPs. Suppression of *AtRanBP1c* expression by antisense technology renders the transgenic roots hypersensitive to auxin, enhances primary root growth, and suppresses lateral root growth (19). This suggests that plant *RanBP*s may have different functions compared to those in animals and fungi. The physiological functions of *NbRanBP1*, a Ran binding protein homolog, in *Nicotiana benthamiana* were investigated by virus-induced gene silencing (VIGS) (20). Gene silencing of *NbRanBP1* caused stunted growth, leaf yellowing, and abnormal leaf morphology. In the later stages of development, *NbRanBP1*-silenced plants exhibited stress responses such as reduced mitochondrial membrane potential, excessive production of reactive oxygen species, and induction of defense-related genes. These results suggested that Ran binding proteins play a key role in stress responses.

The Ran family plays a critical role in nucleocytoplasmic transport during the cell cycle (9). The regulation of expression levels of the *CmRanBP* under abiotic stress conditions have not been studied before. Here, we report for the first time, the isolation and characterization of a cDNA clone encoding a *RanBP* from melon. Comparison of its deduced amino acid sequence with that of previously known *RanBP*s, allowed the identification of new, highly-conserved motifs. Additionally, the gene expression profiles of the *CmRanBP* in different tissues under high temperature stresses were analyzed via semiquantitative RT-PCR.

Materials and methods

Materials

The seed of Galia type Turkish melon was obtained from the Batı Akdeniz Agricultural Research Institute (Antalya, Turkey). The total plant RNA was isolated from 17-day-old root, stem, and leaf tissues using the RNeasy Plant Mini Kit from Qiagen (Qiagen, Valencia, CA, USA). The GeneRacer™ Kit from Invitrogen (Invitrogen, Paisley, UK) was used for the cloning of full-length cDNA. The isolated fragment was cloned using a PCR Cloning Kit (pCR®8/GW/TOPO® TA Cloning® Kit, Invitrogen, Paisley, UK), sequenced, and compared with database sequences using the BLAST program from NCBI. cDNAs were synthesized from RNA samples according to the manual of the RevertAid™, First Strand cDNA Synthesis Kit from Fermentas (Fermentas, Cornaredo, Italy). Sequence similarities were analyzed using the MEGA4 (Molecular Evolutionary Genetics Analysis) (21) and the data from the GenBank database.

Plant culture and sampling

Melon seedlings were grown in plastic pots and irrigated with half strength Hoagland's solution (22) in a growth chamber at 25 °C, with 16 h light periods for 14 days. For heat stress treatments, 14-day-old seedlings were transferred to a growth chamber maintained at 38 °C and 40 °C for 72 h. The root, stem, and leaf tissues from the control and stressed plants were collected, immediately frozen in liquid nitrogen, and stored at –80 °C.

Total RNA isolation

The total RNAs of root, stem, and leaf samples from heat stressed and control melon seedlings were isolated according to the Qiagen RNeasy Plant Mini Kit procedure. RNA quality and quantity were determined photometrically using a single beam spectrophotometer (AlphaTech 3000 Spectrophotometer). The quality of the RNA samples was also verified by 0.8% agarose gel electrophoresis to investigate the RNA integrity.

Isolation of *CmRanBP* gene

A full length *CmRanBP* gene was cloned using the RACE (Rapid amplification of cDNA ends) method with oligonucleotide primers 5'GAATCTACGCTCGAAGATCGACATACC-3' for 5' Race reaction and 5'TTTTCCCCACCCGAAGTCCACATTT-3' for 3' Race reaction. These primers were designed based on the highly conserved amino acid sequences of previously identified plant *RanBP*s. First strand cDNA synthesis was carried out using 10 µg of total RNA. For PCR with specific RanBP primers, 1 µL of cDNA was used A high fidelity PCR system (Invitrogen, Paisley, UK) was used with the following PCR parameters: 2 min template denaturation at 95 °C for 1 cycle, followed by 5 cycles at 95 °C (30 s), 72 °C (1 min 30 s) and 72 °C (1 min 30 s), then 20 cycles at 94 °C (30 s), 57 °C (30 s), and 68 °C (1 min 30 s) with a final 10 min extension step at 68 °C. After amplification of the desired gene site, the PCR product was visualized on a 1% agarose gel and a band of the expected size was excised and purified with the Qiagen MinElute Gel Extraction Kit (Qiagen, Valencia, CA, USA). The purified fragments were cloned into pCR®8/GW/TOPO® vector (Invitrogen) and validated by colony PCR. The inserted cDNA was sequenced with the ABI 310 DNA sequencing system.

Sequence and phylogenetic analysis of the *CmRanBP*

Primer-finding and open reading frame determination for novel melon *RanBP* cDNA sequences were performed using the MEGA4. The protein sequence was deduced and analyzed by the same program. Blastn and Blastp programs were utilized for identification of the homologous sequences for melon *RanBP* cDNA sequence and protein sequence in GenBank (http://www.ncbi.nlm.nih.gov/blast), respectively.

Multiple alignment was performed with the ClustalW (23) in MEGA4. To evaluate phylogenetic relationships among 14 plant species for the gene coding Ran binding protein, a phylogenetic neighbor-joining tree was constructed based on the uncorrected p distance method. Sequences of mammal and yeast RanBPs were used as an outgroup for the phylogenetic tree construction. Reliability of the tree was evaluated by bootstrapping 1000 replicates.

Protein motifs in RanBPs were scanned using a web based program called the MEME Suite (http://meme.nbcr.net/meme4_1/intro.html). MEME analyzes DNA or protein sequences for similarities

and produces a description (motif) for each pattern it discovers. Protein hydrophobicity was analyzed by the DNASIS program as described by Kyte and Doolittle (24).

Expression analysis of the *CmRanBP* gene in different tissues under different heat stress conditions

Expression patterns of the *CmRanBP* gene were investigated using Multiplex PCR in different parts of melon seedlings grown under normal and heat stressed conditions.

To measure the *CmRanBP* gene expression levels, fragments of the *CmRanBP* and *Cm18SrRNA* (used for housekeeping standard) were amplified together in the same PCR reaction. Forward and reverse primers were designed according the *CmRanBP* cDNA sequence. For forward and reverse primers, 5'-TAAACCCTCTTTCATGGC-3' and 5'-CTTCCTTGGATTTTACAGGT-3' were used, respectively. mRNA levels of a gene of interest are often normalized with respect to a housekeeping gene, and its expression is not expected to vary in the samples under different treatments. The *Arabidopsis 18S rRNA* gene sequence (Accession number: X16077) was used to design internal control primers because its expression level is developmentally stable under different environmental conditions and in different tissues (25). The present work confirmed that these primers were able to amplify *C. melo 18S rRNA*, and that this expression was stable across all tissues and conditions tested. The *Arabidopsis 18S rRNA* forward primer 5'-AAACGGCTACCACATCCAAG-3' and the *Arabidopsis 18S rRNA* reverse primer 5'-CCCATCCCAAGGTTCAACTA-3' were used for *18S rRNA* amplification.

To investigate the effects of high temperature on *CmRanBP* gene expression levels in different tissues, 14-day-old melon seedlings were transferred to growth chambers, which were maintained separately at 38 °C and 40 °C. Seedlings were kept in these growth chambers for 72 h. As a control, 14-day-old melon seedlings were held at 25 °C for 72 h. Both stressed and control seedlings were treated with half strength Hoagland's solution during this period. The total RNA was isolated from different tissues including the root, stem, and leaf of stressed and unstressed melon plants as described above. cDNAs were synthesized from DNase-treated RNA samples according to the procedures of the Fermentas RevertAid™ 1st strand cDNA Synthesis Kit. Into DEPC (Diethyl pyrocarbonate-RNase inhibitor)-treated PCR tubes, 2.5 µg of the total RNA samples and 1 µL of oligo (dT) 18 primers (0.5 µg/µL) were added, and the total volume was topped up to 12 µL by the addition of DEPC-treated water. Then the compounds were mixed gently and centrifuged for 5 s. PCR tubes were incubated at 70 °C for 5 min, chilled on ice, and centrifuged for 5 s to collect drops. The tube was placed on ice and the following components were added in the indicated order: 4 µL of 5× reaction buffer, 1 µL of RiboLock™ Ribonuclease Inhibitor (20 U/µL), and 2 µL of 10 mM dNTP mix. Later, they were mixed gently, centrifuged for 5 s and the tubes were incubated at 37 °C for 5 min. After the incubation, 1 µL of RevertAid™ M-MuLV Reverse Transcriptase (200 U/µL) was added and the tubes were incubated at 42 °C for 60 min. Finally, the tubes were incubated at 70 °C for 10 min to stop the reaction and chilled on ice. The target cDNA was amplified by the following procedure: 5 min template denaturation at 95 °C for 1 cycle, followed by 35 cycles at 95 °C (30 s), 51 °C (1 min 30 s), and 68 °C (1 min 30 s), with a final 10 min extension step at 68 °C to extend. The amplification products that belong to different plant parts were visualized on a 1% agarose gel.

Quantification of the expression level of the *CmRanBP* gene

The Scion Image 4.03 image analysis program was used to measure the intensity of bands belonging to the *CmRanBP* and *Cm18SrRNA* genes. First, the actual pixel densities of the defined bands were determined, then the pixel densities of the background were subtracted from the pixel densities of the defined bands, and finally, the densitometric values of the *CmRanBP* gene were normalized with respect to *Cm18SrRNA*. Densitometric values of the control samples were compared with samples treated with heat stresses. Relative expression profiles of the *CmRanBP* gene were evaluated under different heat stress conditions in different tissues.

The experiment was repeated 3 times. The results were expressed as mean ± standard error (ER) from values of 3 independent tests. Groups of data were compared by one-way ANOVA. A difference between means was considered significant at a value of $P < 0.05$.

Results and discussion

Isolation and sequence analysis of the *CmRanBP* gene

The total RNA from different tissues including the root, stem, and leaf was isolated from 17-day-old melon seedlings. A full length *CmRanBP* gene was obtained by Rapid Amplification of cDNA ends from melon leaf cDNA and deposited in GenBank under the accession number EU853459.

As shown in Figure 1, the *CmRanBP* gene is 778 base pair in length. The open reading frame (between position 114 and 707) encodes a protein consisting of 197 amino acids. The predicted polypeptide sequence reveals that the *CmRanBP* encodes an acidic protein with an isoelectric point of 5.06, and a molecular mass of 22.2 kDa. According to the eukaryotic Kozak consensus sequence, the ATG at position 114 is predicted to be the true start codon, with a TATA box located between 28 and 35. A putative polyadenylation signal (ATAAA) is found within positions 723-727.

Characterization and structure analysis of plant *RanBP*s

The predicted polypeptide sequence of the *CmRanBP* shows the highest similarity (above 70%) with *RanBP*s from grape, castor bean, and black cottonwood. Furthermore, the *CmRanBP* is 55%-66% identical to *RanBP*s from other plants including tobacco, mustard, Arabidopsis, and tomato. The consensus sequence of the Ran binding domain

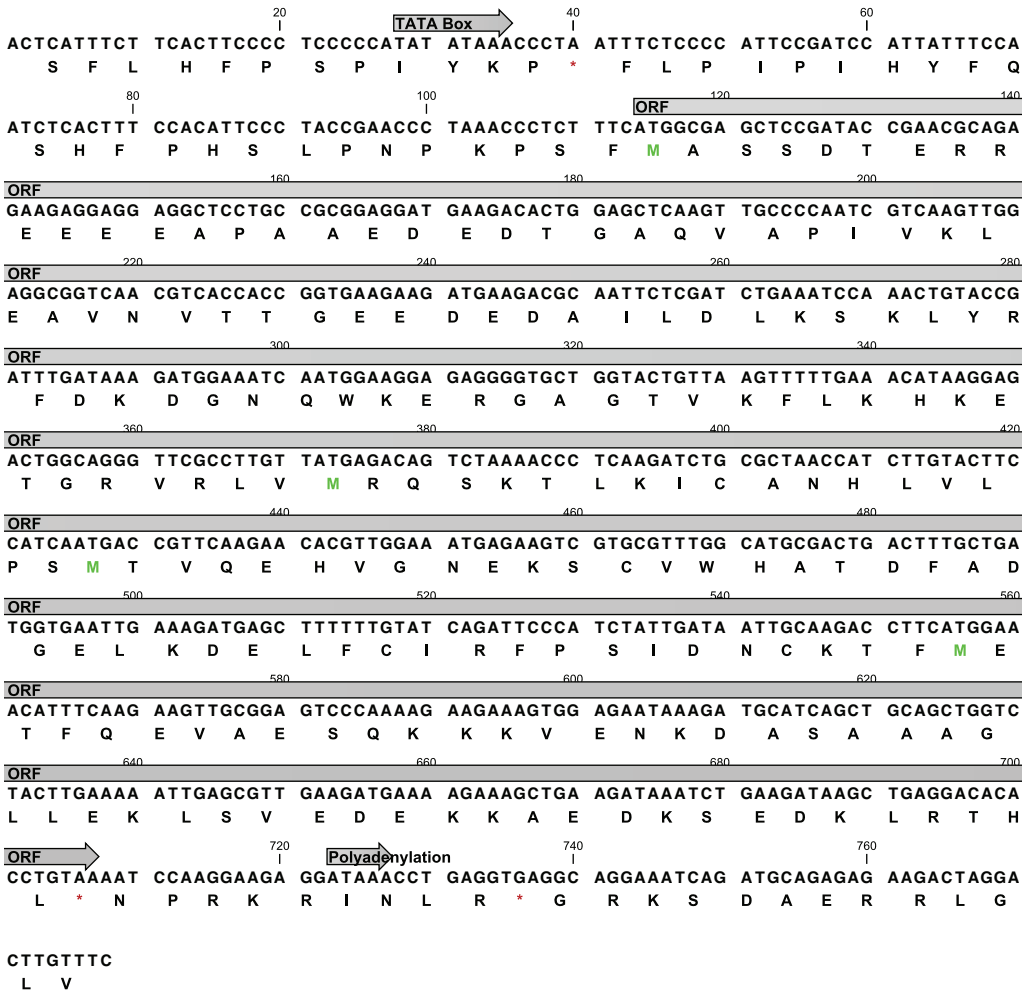

Figure 1. Nucleotide and deduced amino acid sequences of melon *RanBP*. TATA box and polyadenylation site start at position 28 and 723 bp, respectively, and are shown as black boxes. The GenBank accession number of melon RanBP is EU853459.

(RanBD), located between amino acid residues of 30 and 160 in plant species, and 5 highly conserved motifs are outlined in Figure 2. These conserved motifs could play a role in interactions with Ran proteins. In addition to these highly conserved motifs, melon RanBP contains a leucine-rich nuclear export signal (NES) located at a position of L_{174}, L_{177}, and V_{179}, which is not present in the C-terminus of RanBPs from fungi and protozoa. NES is required for the cytoplasmic localization of a functional RanBD (26).

The hydropathy profiles of *CmRanBP*, *VvRanBP*, *RcRanBP*, and *PtRanBP*, as representatives of the family showing the highest homology, were predicted by the DNASIS program with a window size of 9 amino acid residues (Figure 3). Kyte-Doolittle is a

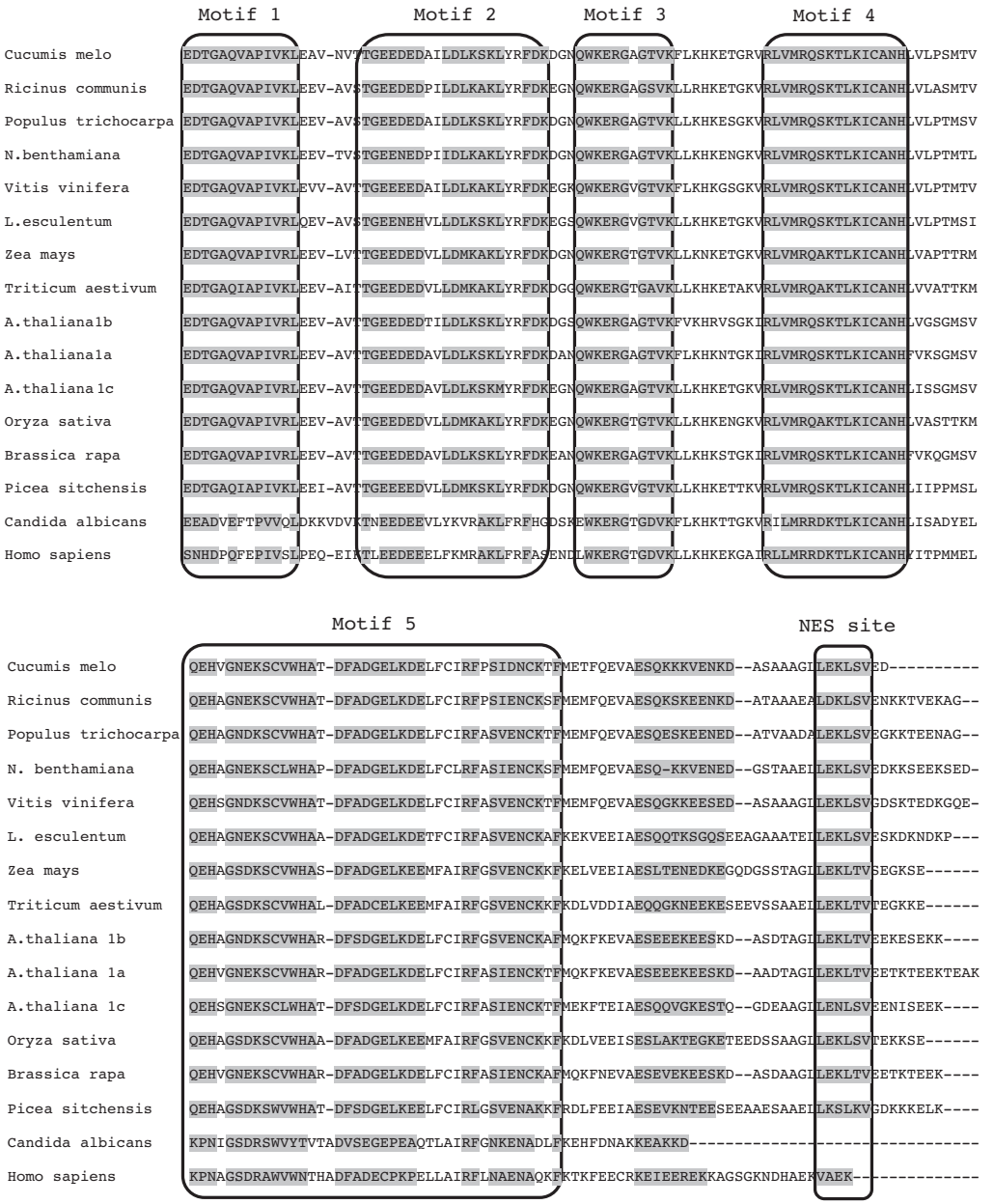

Figure 2. Alignment of the deduced amino acid sequence of *CmRanBP* with 13 RanBPs from different plant species. Residues that are conserved between the sequences are black or light gray based on the degree of conservation.

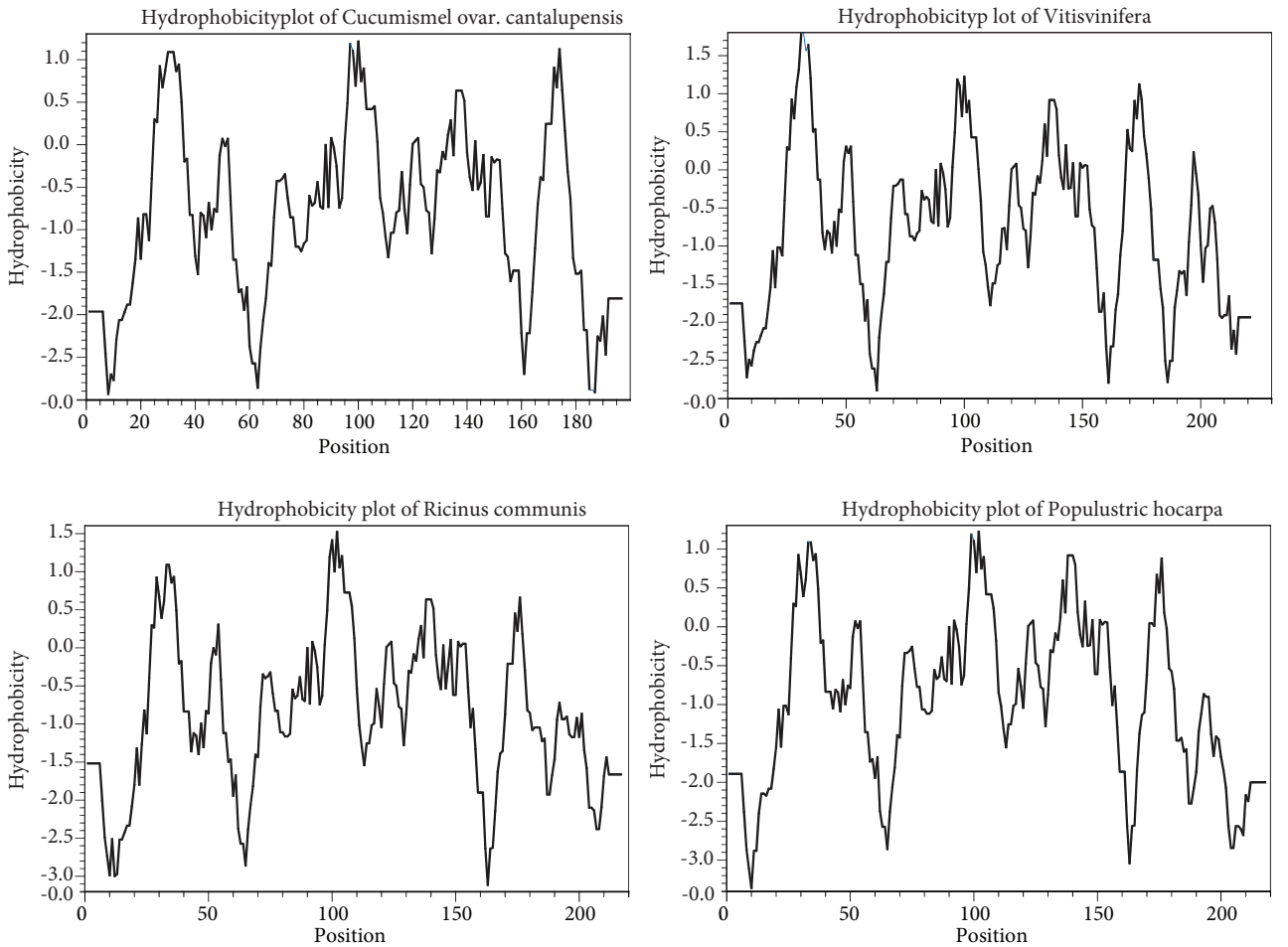

Figure 3. Hydropathy profiles of *CmRanBP* (*Cucumis melo*), *VvRanBP* (*Vitis vinifera*), *RcRanBP* (*Ricinus communis*), and *PtRanBP* (*Populus trichocarpa*). Hydrophobicity was analyzed by the DNASIS program with a window size of 9 amino acid residues. Hydrophilic domains are below the zero line.

widely applied scale for delineating the hydrophobic character of a protein. Regions with values above zero are hydrophobic in character whereas hydrophilic domains are below the zero line. According to Kyte-Doolittle, when the window size is 9, strong negative peaks indicate possible surface regions of globular proteins. All of the proteins considered exhibited this pattern. Hydropathy analysis also revealed that all of the RanBPs were highly conserved in the middle region of their amino acid sequence, which included the 5 conserved motifs mentioned above. However, the N- and C-termini exhibited a very low similarity across the different RanBPs.

Construction of the phylogenetic tree of plant *RanBP*s

RanBP sequences from 14 different plant species were selected for construction of a phylogenetic tree to determine the evolutionary relationship among *RanB*Ps in plants. The amino acid sequence of the *CmRanBP* was aligned with related sequences from grape, castor bean, black cottonwood, sitka, tobacco, mustard, Arabidopsis, rice, corn, wheat, tomato, yeast, and human.

Multiple sequence alignment revealed a high similarity and sequence conservation in the Ran-

binding domain of all of the RanBPs from the different classes of plant species. However, a much lower homology was found in the N- and C-terminal regions, flanking this domain consistent with the hydropathy analysis results.

Within the RanBD, 5 highly conserved domains and 1 Ran binding motif were found in all members of the *RanBP* gene family from various species according to the MEME Suite.

A phylogenetic tree was constructed from the available protein sequences of 14 different plant RanBPs, with yeast and mammal sequences being used as an outgroup to establish a proper branching order. The tree architecture reflected the divergence between both gymnosperm-angiosperm and monocot-dicot plant species. According to the neighbor-joining tree (Figure 4), the *RanBP* from *Picea sitchensis* branched off earlier than all the angiosperm species. A further split occurred between the monocotyledon and dicotyledon species. The *CmRanBP* gene was placed within the dicot clades.

Expression analysis of the *CmRanBP* gene

To examine the expression pattern of the *CmRanBP* in different melon tissues, a semiquantitative RT-PCR analysis was performed using *CmRanBP*-specific primers that amplified the expected 600 bp fragment. The *18S rRNA* expression level (250 bp fragment) was also measured as a control. *CmRanBP* transcripts were detected at similar levels in all of the control tissues examined, including the root, leaf, and stem (Figure 5). Following a rise in temperature to 38 °C and 40 °C, the *CmRanBP* transcript level significantly ($P < 0.05$) increased in the root, leaf, and stem tissues (Figure 6). When compared to all the control tissues (root, leaf, and stem), an increase of about 2-fold in the CmRanBP gene expression was observed in all of the tissues that were maintained at 38 °C and 40 °C for 72 h.

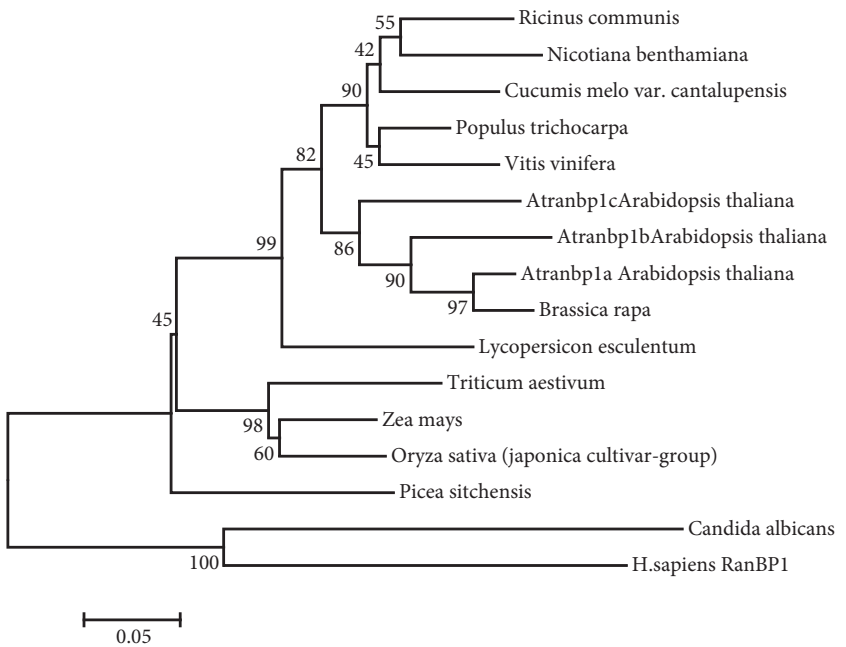

Figure 4. Phylogenetic relationships between Ran binding proteins from 14 different plant species. The neighbor-joining phylogenetic tree was constructed based on uncorrected p distance. Bootstrap values are shown on the branches. Their GenBank accession numbers are: *Arabidopsis thaliana* AtRanBP1a (Accession number CAA66045), AtRanBP1b (CAA66046), AtRanBP1c (AAB38776); *Brassica rapa* (ABB97039); *Candida albicans* (AAG43107); *Homo sapiens* (NP_002873); *Lycopersicon esculentum* (AAK53813); *Nicotiana benthamiana* (ABS50234); *Oryza sativa* (NP_001055275); *Picea sitchensis* (ABK24143); *Populus trichocarpa* (XP_002313984); *Ricinus communis* (EEF45739); *Triticum aestivum* (AAZ41378); *Vitis vinifera* (XP_002262903); and *Zea mays* (ACG33387).

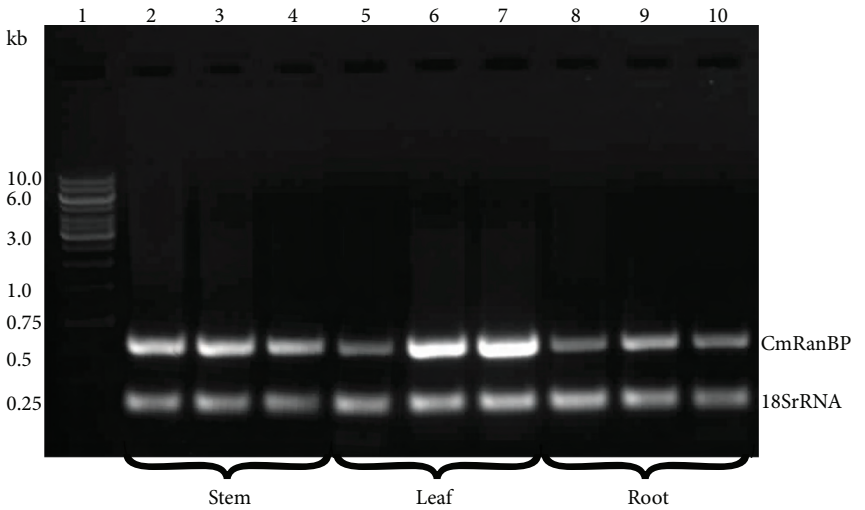

Figure 5. Agarose gel electrophoresis of the *CmRanBP* gene isolated from root, leaf, and stem tissues. 1: DNA marker (Fermentas GeneRuler™ 1 kb DNA Ladder, 250-10,000 bp) 2: 25 °C (Control stem tissue) 3: 38 °C 4: 40 °C 5: 25 °C (Control leaf tissue) 6: 38 °C 7: 40 °C 8: 25 °C (Control root tissue) 9: 38 °C 10: 40 °C

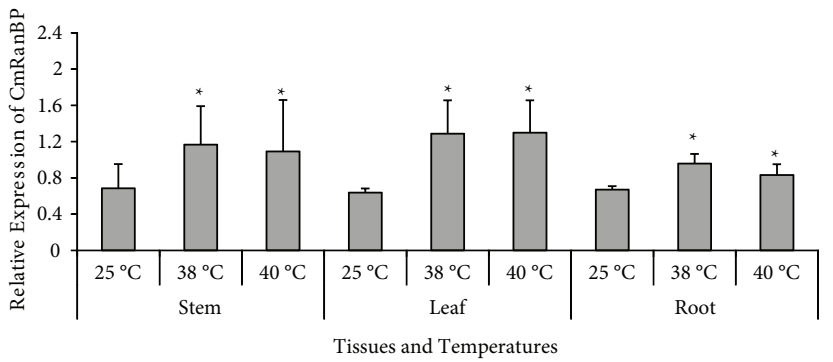

Figure 6. Quantified analysis of the *CmRanBP* gene expression. Results were generally expressed mean ± standard error (ER) from values in 3 independent tests. Groups of data were compared by one-way ANOVA, $^{*}P < 0.05$, n = 3.

Evaluation of *CmRanBP* gene structure

Ran is a Ras-like nuclear small GTP-binding protein, regulating receptor-mediated transport between the nucleus and the cytoplasm (14). RanBPs are accessory proteins that regulate Ran activity by binding to the Ran protein (27). Therefore, understanding the structures and functions of Ran and RanBP will be useful for further studies. We isolated a *CmRanBP* and found highly conserved motifs that represent binding sites for Ran. The conserved motifs in *RanBP* genes suggest that it might be a common feature of this gene family in plants, and it also supports the idea that this *RanBP* gene family plays a significant role in plant growth and physiological development. A previous study showed that silencing an *Arabidopsis AtRanBP1c* gene by antisense technology caused auxin hypersensitivity during root development by blocking mitotic progression in the primary and lateral roots (19). Additionally, *Nicotiana benthamiana NbRanBP1*

silenced plants showed stress responses such as reduced mitochondrial membrane potential, excessive production of reactive oxygen species, and induction of defense-related genes (20).

The hydropathy profiles of the *CmRanBP* exhibit strong negative peaks indicating possible surface regions of globular proteins. The globular nature of Ran-binding protein may facilitate transportation of molecules through membranes. This suggests that Ran family members play a central role in nucleocytoplasmic transport.

According to the phylogenetic tree, plant RanBPs found in GenBank form 2 main groups, representing gymnosperm and angiosperm. Although 2 distinct groups exist in the phylogenetic tree, the *CmRanBP* exhibits above 50% similarity with the *PsRanBP*. This implies that Ran binding domains that participate in transport processes are highly conserved in all plant species.

Gene expression profiles of the *CmRanBP*

The evaluation of gene expression levels of the *RanBP* gene under various heat stress conditions can play a critical role in understanding the effect of heat on melon plant growth. Although the *CmRanBP* gene was expressed constitutively with similar levels in the control tissues including the root, leaf, and stem, the *CmRanBP* expression profiles dramatically changed in these tissues following the exposure of the plant to high temperatures for 72 h. Haizel et al. (16) reported that the *At-Ran* and *AtRanBP* genes were expressed in all tissues examined, including leaves, stems, roots, and flowers, with the highest level of expression in meristematic tissues such as the shoot and the root apical meristem. Furthermore, those genes are coordinately expressed at all stages of the Arabidopsis development (16). Tian et al. (14) recently demonstrated that the wheat (*Triticum aestivum*) *TaRanBP* gene is expressed similarly in its leaf, stem, and root tissues. In our study, the *CmRanBP* gene expression profile observed in control tissues is consistent with these previous results. However, when the plants were transferred to a growth chamber with elevated temperatures for 72 h, the levels of *CmRanBP* transcripts were increased by about 2-fold in all of the tissues, including the root, leaf, and stem. This suggests that the *CmRanBP* may play a role in the physiological processes associated with heat stress response. Although there are some studies related to expression profiles of *RanBP* genes from different plant species (20,28), this is the first study that examines the effect of heat stress on expression levels of the gene responsible for the synthesis of RanBP in melon.

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