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Isolation, characterization and expression analysis of the GDP dissociation inhibitor protein gene *MiRab-GDI* from *Mangifera indica* L

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ABSTRACT

Rab guanosine nucleotide dissociation inhibitor (Rab-GDI) proteins are important Rab GTPase effectors, which control the cycling of small GTPases between membrane and cytosol byinteracting with the prenylated GDP bound small GTPases. However, little is known about the function of Rab-GDIs in fruit plants. In this study, we present the cloning and characterisation of a putative *MiRab-GDI* (GenBank accession no. KF768565) from *Mangifera indica*. The gene encoded a 444 amino acid protein with a molecular weight of 49.75 kDa and a theoretical isoelectric point of 5.48. The deduced amino acid sequence exhibited high homology with *Nicotiana tabacum* (92% similarity) and contained five structurally and functionally defined sequence conserved residues (SCRs). SDS–PAGE and Western blot analysis indicated that recombinant MiRab-GDI was successfully expressed in *E. coli* and the molecular weight of the solute protein was the same as the predicted value. Real-time RT–PCR analysis demonstrated that *MiRab-GDI* was ubiquitously expressed in various plant tissues and organs at different levels. The expression of *MiRab-GDI* was down-regulated in the early stages of fruit development and up-regulated during later stages of fruit ripening. In addition, it could be induced by various treatments such as cold, NaCl, drought, abscisic acid (ABA), salicylic acid (SA), and hydrogenperoxide (H_2O_2). These results provide insights into the role of the *MiRab-GDI* gene in fruit ripening and abiotic stresses in the mango plants.

1. Introduction

Rab GTPases are critical regulators of endocytic and secretory pathways in eukaryotic cells (Novick and Hutagalung, 2011). Rab GTPases regulate membrane trafficking by cycling between the inactive GDP-bound and active GTP-bound states. These two states allow Rab GTPases to function as molecular switches that control diverse biochemical pathways via interactions with effectors (Grosshans et al., 2006). Three classes of effectors of Rab GTPase proteins have been found and characterized in animals and yeast: GDP/GTP exchange factors (GEFs), GTPase activating proteins (GAPs) and GDP-dissociation inhibitors (GDIs) (Schmidt et al., 2007). GEFs promote the exchange of GDP for GTP, GAPs stimulate intrinsic GTP hydrolysis activity, and GDIs regulate both the nucleotide state and the subcellular localization of Rab proteins. The cycling of small GTPases between membrane and cytosol is controlled by GDIs interacting with the prenylated GDP bound small GTPases (Nibau et al., 2006; Schmidt et al., 2007). Unlike other Rab effectors, most Rab-GDI isoforms recognize a broad range of Rab species in vitro, and more than one Rab-GDI isoforms can associate with a single Rab member. For example, the cytosolic Rab4 is simultaneously associated with both GDI-1 and GDI-2 under basal conditions (Shisheva and Czech, 1997).

Rab-GDIs play a critical role in the recycling of Rab family GTPases that regulate the formation, targeting and fusion of vesicles involved in membrane trafficking. Rab-GDIs form a complex with prenylated Rab proteins that are in a GDP-bound state in the cytosol and mediate the delivery of these Rabs to their target membranes during vesicle formation. Subsequnetly, Rab-GDIs mediate Rab retrieval from the membrane after the catalytic cycle is completed (Ignatev et al., 2008).

Rab-GDIs have been well characterized in yeast and animals. There are more than 60 different Rab GTPases, but only five Rab-GDI isoforms have been identified in mammalian cells (Alory and Balch,

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2001).There is only one *GDI* gene (*SEC19*/*GDI1*) in yeast and more than five in mice (Janoueix-Lerosey et al., 1995). The GDI1-related vesicle transport system is required for the secretion of Zn ions and ameliorates Zn toxicity in yeast cells, allowing yeast to survive in the presence of toxic Zn (Ezaki and Nakakihara, 2012). Several Rab GDIs have been isolated from animals including humans, bovines, rats, and *Drosophila*. These studies have indicated that Rab GDIs are involved in vesicular trafficking in diverse types of cells. In contrast, only a few Rab GDI that share homology with other members of the GDI family have been reported in plants. In *Arabidopsis*, both AtGDI1 and AtGDI2 can interact with Ara4 in yeast ypt1 mutant cells and complement the yeast sec19 (gdi1) mutation (Ueda et al., 1996; Zarsky et al., 1997; Ueda et al., 1998; Ezaki et al., 1999). A tobacco NtGDI1 complemented the Al-sensitive phenotype exhibited by the yeast sec19 mutant and provided basal Al resistance in yeast through the export of Al ions (Ezaki et al., 1999; Ezaki et al., 2005). Over-expression of *NtGDI1* in transgenic *Arabidopsis* plants activated an Al efflux system that protected *Arabidopsis* against Al toxicity (Kim et al., 1999; Ezaki and Ezaki 2000). *OsGDI1* and *OsGDI2* were isolated from a cDNA library of fungal-elicitor-treated rice and can rescue the Sec19 mutant of *Saccharomyces cerevisiae*, which is defective in vesicle transport (Kim et al., 1999).

However, knowledge about the function of Rab-GDI on expression patterns or responses to exogenous signaling molecules is limited in plants. In this study, we describe a novel *MiRab-GDI* gene cloned from mango plants, which is widely cultivated in southern China. We describe the expression patterns of this gene in different organs, during fruit development, and after treatment with various exogenous signaling molecules.

2. Materials and methods

2.1. Plant materials

Young leaves, old leaves, young stems, old stems, flowers, and fruits from 20, 30, 40, 50, 60, 70 and 80 d after flowering samples were collected from 11-year-old mango trees (*M. indica* L. cv. 'SiJi') in an orchard in Guangxi University, Southeastern China. Each sample was immediately frozen in liquid nitrogen and stored at −80 °C.

All stress treatments were conducted on one-year-old *M. indica* L. cv. 'SiJi' grafted plants (on rootstock 'Tu' mango plants) grown in soil-filled pots under normal conditions. For low temperature stress, the plants were transferred to growth chambers adjusted to 4 °C with 16 h light and 8 h dark photoperiod at a light intensity of 400 μmol m^{-1} s^{-1}). Salinity and drought treatment were conducted by watering each pot with 300 mM NaCl and 300 g L^{-1} polyethylene glycol (PEG-6000). Plants that were not subjected to stress treatments served as control group under the same growth conditions. All tested and control group samples were collected at 0, 24, 48, and 72 h after treatments, respectively. The signal molecules treatments involved spraying plants with 100 μM abscisic acid (ABA), 1 mM salicylic acid (SA), and 1 mM hydrogen peroxide (H_2O_2) solutions, respectively. Plants that were not subjected to stress treatments served as controls and were kept under the same growth conditions. Samples were collected at 0, 0.5, 1, 2, 4, 8, 12 and 24 h after treatments and stored at −80 °C until RNA was isolated.

2.2. RNA extraction and cDNA synthesis

Total RNA was isolated from the samples of mango mentioned above using Quick RNA Isolation Kit for plant tissues rich in polyphenols and polysaccharides (Waryong, China). Then, the RNA was treated with DNase I digestion (Waryong, China) to eliminate potential DNA contamination. The integrity of the RNA samples was assessed by gel electrophoresis on 1% agarose and ultraviolet spectrophotometer (Eppendorf, Germany) analysis. High-quality total RNA (1 μg) from the samples was used to synthesize first-strand cDNA using the M-MLV reverse transcriptase (Takara, China) with primer AUP1 (5′-GGCCACGCGTCGACTAGTACTTTTTTTTTTTTTTTT-3′). The quality and concentration of RNA and first-strand cDNA were determined by 1.2% agarose gel electrophoresis and ultraviolet spectrophotometer (Eppendorf, Germany) analysis.

2.3. Cloning and sequence analysis

The *MiRab-GDI* cDNA fragment was amplified by cDNA-SCoT (Start codon targeted polymorphism) analysis in our previous study and only lacked the 5′ end sequence. A modified 5′ RACE (rapid-amplification of cDNA ends) was performed in this study to amplify the 5′ end fragment (Luo et al., 2011). First-strand cDNA was purified, and homopolymer dC-tails were added to the 3′ ends of the cDNA by terminal deoxynucleotidyl transferase with dCTP (Takara, China). Two nested gene-specific primers, MiGDId1 (5′-CATTCCCTCGTGTGTTTTGG-3′) and MiGDId2 (5′-CACGCACAAGACCTCCATTT-3′) were designed based on the known sequence of *MiRab-GDI*. Adaptor primers AP1 (5′-AAGCAGTGGTATCAACAGAGTACGCGGGGGGGGGG-3′) and AP2 (5′-AAGCAGTGGTATCAACGCAGAGT-3′) were designed to conduct nested PCR with the gene-specific primers. The first 5′ RACE reaction was performed using the tailed cDNA as a template with the MiGDId2 and AP1 primers. The products of the first 5′ RACE were diluted 40 times and used as template for the second 5′ RACE amplification with the MiGDId1 and AP2 primers. After two rounds of nested PCR were performed with 5′ RACE, the products of the two reactions were verified by agarose gel electrophoresis, and the target fragments were selected by size based on the nested primers. Subsequently, the product was cloned and sequenced. After obtaining the 5′ end fragments, the full-length *MiRab-GDI* sequence was amplified by RT-PCR. The PCR was run in a T-Professional thermocycler (Biometra, Germany) under the following conditions: initial denaturation of 4 min at 95 °C, then 35 cycles of 40 s at 94 °C, 40 s at 58 °C, and 1 min 40 s at 72 °C, and a final extension of 10 min at 72 °C.

Identification of nucleotide sequences was conducted with the NCBI Blast program [http://www.ncbi.nlm.nih.gov/BLAST]. The conserved domains were analyzed with InterProScan [http://www.ebi.ac.uk/Tools/pfa/iprscan]. Automated protein structure homology modeling was performed in Phyre2 [http://geno3d-pbil.ibcp.fr]. The theoretical isoelectric point (p*I*) and molecular weight (*Mw*) for mature peptides were calculated using the PeptideMass program [http://us.expasy.org/tools/peptide-mass.html]. Multiple sequence alignments were performed on Clustalx 2.0 and GenDoc 2.7, and a phylogenetic tree was constructed using the neighbor-joining (NJ) method in MEGA 5.2.

2.4. Construction, expression, and purification of recombinant protein

Recombinant MiRab-GDI protein was produced as follows. The coding region of *MiRab-GDI* was PCR-amplified, digested with restriction enzymes, and ligated into the pET-30a vector to generate pet-MiGDI. The PCR primers used for the construction of this vector were pet-GDIF2 (5′-CGGAATTCATGGATGAAGAGTATGA-3′) and pet-GDIR2 (5′-GCGAGCTCTTCTTCTGCAGCGCTGG-3′). The in-frame fusion between the histidine tag and MiRab-GDI was verified by nucleotide sequencing. A single positive clone was picked with a sterilized toothpick and transferred into 10 mL of LB liquid medium (with Kan 50 mg L^{-1}) culture liquid. The culture was placed in a shaker at 37 °C with a constant speed of 200 r min^{-1}. IPTG was