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Two translationally controlled tumor protein (TCTP) genes from *Hevea brasiliensis* play overlapping and different roles in development and stress response

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ABSTRACT

Translationally controlled tumor protein (TCTP) is a highly conserved protein and performs vital roles in regulating general growth and development in eukaryotes. Rubber tree (*Hevea brasiliensis*) harbors two *TCTP* genes, *HbTCTP* and *HbTCTP1*, but the individual functions of them were unknown in plant. Here, sequence analysis displayed that *HbTCTP* and *HbTCTP1* had the same number of 5′-terminal oligopyrimidine tract (5′-TOP) motifs in the 5′-untranslated region (UTR). However, the number of AUUUA motifs in the 3′-UTR was different between two *TCTPs*. *HbTCTP* and *HbTCTP1* indicated different expression profiles in phloem and its exudate under ethylene treatment, suggesting that functional divergence likely occurred between two *TCTPs* in rubber tree. Moreover, *HbTCTP and HbTCTP1* had different effect on adventitious shoot differentiation from leaf explants during transformation. Overexpressing *HbTCTP* or *HbTCTP1* in tobacco enhanced the tolerance to H_2O_2-mediated oxidative stress and alleviated cell death induced by tunicamycin, suggesting that *HbTCTP* and *HbTCTP1* played overlapping roles in the oxidative stress resistance and anti-apoptosis. Our results provide important insights into function and characteristics of *HbTCTP* and *HbTCTP1*.

1. Introduction

Translationally controlled tumor protein (TCTP) is a highly conserved and ubiquitously expressed protein in eukaryotes. Its expression is regulated at both translational and transcriptional levels in response to a wide range of extracellular signals and cellular conditions (Bommer and Thiele, 2004). TCTP is a multifunctional protein involved in diverse processes such as cell proliferation, cell division and growth, cell cycle progression, malignant transformation, microtubule organization, ion homeostasis, stress protection and apoptosis (Bommer and Thiele, 2004; Bommer, 2012; Amson et al., 2013). There are variable numbers of *TCTP* genes in different organisms, while plants generally harbor one or two *TCTP* genes (Gutiérrez-Galeano et al., 2014). Recent studies have indicated that *TCTPs* possess a division of function and may be perform non-overlapping or partially overlapping functions. Based on the probable functions and predicted structures, *TCTPs* are divided into *AtTCTP1*-like and *CmTCTP*-like clades. Gutiérrez-Galeano et al. (2014) supposed that the functions of *TCTP* members might be inferred from their corresponding clades.

Currently, several *TCTP* genes have been obtained from different plant species. However, studies on *TCTP* function have focused mostly on human and animals. The biological function of plant *TCTP* is not yet well characterized. Plant *TCTP* expression is affected by a variety of abiotic stresses including darkness, H_2O_2, low temperature, salinity, drought, heavy metal and aluminum (Li et al., 2013; Gutiérrez-Galeano et al., 2014; Deng et al., 2016), biotic stress including pest, pathogen and virus (Gupta et al., 2013; Bruckner et al., 2017; Zhang et al., 2014), and hormone including ABA, methyl jasmonate, auxin and ethylene (Gutiérrez-Galeano et al., 2014; Tao et al., 2015). Moreover, plant *TCTPs* have been associated with growth and development, such as pollen tube growth and male gametophyte maturation, endosperm development, root formation and development, regulation of stomatal

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closure, fruit ripening and senescence, photoperiodism and flowering, and symbiotic nodulation (Berkowitz et al., 2008; Zhang et al., 2011; Gutiérrez-Galeano et al., 2014; Toscano-Morales et al., 2016; Chou et al., 2016). Interestingly, either *CmTCTP* or *AtTCTP2* harbored by *Agrobacterium rhizogenes* was capable of inducing whole plant regeneration in tobacco. However, *AtTCTP1* couldn't induce regeneration, suggesting that *TCTPs* had a non-redundant function in plant regeneration (Hinojosa-Moya et al., 2013; Toscano-Morales et al., 2015). Both *CmTCTP* and *AtTCTP2* were involved in long-distance movement of phloem through a non-cell autonomous manner (Hinojosa-Moya et al., 2013; Toscano-Morales et al., 2014). Overexpressing *AtTCTP1* significantly reduced cell death triggered by different effectors in tobacco (Hoepflinger et al., 2013).

As the only commercial source of natural rubber (*cis*-1, 4-polyisoprene), rubber tree (*Hevea brasiliensis*) is a perennial plant belonging to the Euphorbiaceae family. There are two *TCTP* genes, *HbTCTP* and *HbTCTP1*, in rubber tree (Liang et al., 2009; Li et al., 2013). The gene structure and molecular markers of *HbTCTP* were analyzed (Deng et al., 2012, 2016). The subcellular location and interacting proteins of rubber tree TCTPs were identified (Deng et al., 2016; Wei et al., 2016). Additionally, rubber tree TCTPs had antioxidative activity in metal-catalyzed oxidation (MCO) system and calcium-binding activity. The expression patterns of *HbTCTP* and *HbTCTP1* were associated with tapping panel dryness (TPD), hormone and stress responses in rubber tree, but their exact functions are not well known (Li et al., 2013; Deng et al., 2016). In the present study, 5′- and 3′-UTR and genomic structure characteristics of rubber tree *TCTPs* were analyzed in detail. *HbTCTP and HbTCTP1* were regulated by ethylene in the phloem and its exudate, suggesting that they might be associated with long-distance movement in rubber tree. Interestingly, *HbTCTP and HbTCTP1* affected the differential ability of adventitious shoot from tobacco leaf explants during transformation. In addition, we validated that the overexpression of *HbTCTP* or *HbTCTP1* resulted in enhanced oxidative tolerance and alleviated cell death triggered by tunicamycin in transgenic tobacco. Our results suggest that there are overlapping and different functions between two rubber tree *TCTPs*.

2. Materials and methods

2.1. Plant materials

Reyan7-33-97, a high-yielding rubber tree clone, was planted at the experimental farm of Chinese Academy of Tropical Agricultural Sciences. Latex and leaves were collected from nine-year-old rubber trees regularly tapped on the s/2 d/3 system. One-year-old epicormic shoots of rubber tree were used as experimental materials. Barks of extension unit 3 were treated with 1% ethylene and distilled water, respectively. After 24 h treatment, the tissue containing primary laticifer and its outer layers were scrapped with a sharp blade according to bark structure of rubber tree described by Tian et al. (2015), and then the surface of barks was rinsed with distilled water. Subsequently, the secondary phloem and its exudate were collected by scarping with a sharp blade.

2.2. RNA and DNA extraction

Total RNA from latex, phloem and its exudate were isolated according to Xu's method (Xu et al., 2010). The genomic DNA from leaves was prepared with the CTAB method (Murray and Thompson, 1980).

2.3. RACE

Using total RNA from latex as template, the 5′/3′-RACE-ready cDNA was synthesized according to the SMART™ RACE cDNA amplification kit (Clontech, USA). 5′-RACE PCR was performed with primers: HbTCTP-5RACE-GSP (5′-CCCCTTGAACAACCCACTTC-3′) and HbTCTP-5RACE-NGSP (5′-CCTTGTATGGGAACGAGTCA-3′). 3′-RACE PCR was performed with primers: HbTCTP-3RACE-GSP (5′-GCTGGACGAGGAG AAGCAAG-3′) and HbTCTP-3RACE-NGSP (5′-CATGCATGATGATGGCA GTC-3′). The primary PCR was performed using universal primer A mix (UPM) and gene-specific primers. The secondary PCR was carried out using nested universal primer A (NUP) and nested gene-specific primer. All PCR products were cloned into the pMD18-T vector (Takara, Japan) and sequenced.

2.4. Isolation of the genomic and promoter sequences of HbTCTP1

LVXX01000078.1, a genome shotgun sequence of rubber tree, has high homology to the cDNA sequence of *HbTCTP1*. Based on LVXX01000078.1, the gene specific primers HbTCTP1-g-F (5′-ATGCT CGTTTATCAGGATCT-3′) and HbTCTP1-g-R (5′-GTGAGACTCCCTTCTT AACAC-3′) were designed and applied to amplify the genomic sequence of *HbTCTP1*. The promoter sequence of *HbTCTP1* was confirmed by PCR amplified with the gene specific primer HbTCTP1-p-F (5′-AATCT GCTGTGAACAGATCA-3′) and HbTCTP1-p-R (5′-CATCCAGCTAAAACA CCAGA-3′). All PCR products were cloned into the pMD18-T vector (Takara, Japan) and sequenced. The promoter *cis*-acting elements was analyzed by online PLACE databases (https://sogo.dna.affrc.go.jp/cgi-bin/sogo.cgi?lang=en&pj=640&action=page&page=newplace)

2.5. Real-time RT-PCR

The RNA samples from phloem and its exudate were digested with DNase I (Takara, Japan), and first-strand cDNA was synthesized using ReverAid first strand cDNA synthesis kit (Thermo Scientific, USA). The gene-specific primers of *HbTCTP* and *HbTCTP1* were designed according to Li et al. (2013) and Deng et al. (2016). A mitosis protein gene (*YLS8*) (GenBank accession: HQ323250) was used as the internal reference according to Li et al. (2011). Real-time RT-PCR was performed with the fluorescent dye SYBR Premix Ex Taq (Takara, Japan) and CFX96 Touch™ Real-Time PCR Detection system (Bio-Rad, Hercules, CA, USA). PCR was carried out as follows: 94 °C for denaturation, followed by 45 cycles of 94 °C for 5 s, 60 °C for 20 s, and 72 °C for 20 s. The relative expression values of the genes were calculated using the $2^{-D\Delta CT}$ method (Livak and Schmittgen, 2001) in the CFX Manager Software 3.0. All real-time RT-PCR experiments were reproduced with three biological replicates, and the values are presented as the mean ± S.D.

2.6. Plant transformation and generation of transgenic plants

The full-length ORFs of *HbTCTP* (GenBank accession: FJ156098) and *HbTCTP1* (GenBank accession: JN200814) were separately inserted into the binary vector pCAMBIA1301 via the *Nco* I and *Bst*E II sites. The recombinant plasmids under the control of the CaMV 35S promoter were introduced into *Agrobacterium tumefaciens* strain AH109. Transformation of tobacco (*Nicotiana benthamiana*) was performed using an *Agrobacterium*-mediated leaf disc method (Horsch et al., 1985). The transgenic seeds were selected on MS medium containing 40 mg/L hygromycin. The hygromycin-resistant T_1 seedlings DNA were isolated using CTAB method (Murray and Thompson, 1980). Putative transgenic plants were confirmed by gDNA PCR analysis with CaMV 35S promoter specific primer (5′-GAAGGTGGCTCCTACAAATG-3′) and reverse gene-specific primers (5′-GTCCAGCTTAGGTGTCAACAAC-3′ for *HbTCTP* or 5′-GCCCATATGCAAGGTACAGA-3′ for *HbTCTP1*). The hygromycin-resistant T_2 seedlings RNA were isolated using Trizol reagent. Overexpression of *HbTCTP* or *HbTCTP1* in T_2 tobacco lines was further determined by semi-quantitative RT-PCR analysis. *NbEF1α* (GenBank accession: AY206004) was used as the internal reference. The primer pairs used were: *HbTCTP* (F 5′-ATGTTGGTCTATCAGGATTTGC-3′ and R 5′-GCATTTGACCTCCTTCAAAG-3′); *HbTCTP1* (F 5′-CAGGATCTTCTCA CAGGTGA-3′ and R 5′-GCCCATATGCAAGGTACAGA-3′); *NbEF1α* (F