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Molecular Characterization and Activity Analysis of Promoters from Two Cucumber Translationally Controlled Tumor Protein Genes (*CsTCTPs*)

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Aims: The aim of the paper was to isolate and characterize the promoters of two cucumber TCTP genes (*CsTCTP1* and *CsTCTP2*) and evaluate their active domains.

Study Design: *CsTCTP1* and *CsTCTP2* promoter activity were analyzed under treatments with different exogenous hormones.

Place and Duration of Study: In 2017, these experiments were conducted in College of Bioscience and Biotechnology of Shenyang Agricultural University (Lab 240).

Methodology: *CsTCTP*_{pro}::*GUS* constructs were generated by using double digests method. Transient expression was mediated by *Agrobacterium tumefaciens* GV3101. Histochemical and Fluorometric GUS Assays were follow by the biochemical method.

Results: Bioinformatics analysis revealed some hormone- and defense-related response elements. Histochemical and fluorometric GUS assays demonstrated that the 0.7-kb *CsTCTP1* promoter (proT1-0.7kb) and 0.7-kb and 1.3-kb *CsTCTP2* promoters (proT2-0.7kb and proT2-1.3kb) had strong transcriptional activity. In addition, we used exogenous hormones (abscisic acid [ABA], salicylic acid [SA], and ethylene [ETH]) for treatment. The results showed that proT1-0.7kb and

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proT2-1.3kb activity were upregulated in the ABA treatment group, suggesting that these promoter sequences may contain ABA-related response elements. However, in the SA and ETH treatment groups, the activity of all the promoter fragments of *CsTCTP1* and *CsTCTP2* declined to different degrees, suggesting that SA and ETH may have negative regulatory effects on *CsTCTP1* and *CsTCTP2* promoters.

Conclusion: Taken together, these results suggest that the proT1-0.7kb promoter of *CsTCTP1* and proT2-1.3kb promoter of *CsTCTP2* may contain ABA-related response elements, and SA and ETH may have negative regulatory effects on the *CsTCTP1* and *CsTCTP2* promoters. This study will help to further understand the expression patterns and the regulatory mechanism of gene transcriptional regulation.

Keywords: Bioinformatics; CsTCTP; fluorometric GUS assay; histochemical assay; promoter.

1. INTRODUCTION

Correct and efficient gene expression ensures the adaptation of plants to their environment. Gene expression is regulated by noncoding elements over the lifespan of a plant. Promoters are DNA sequences that specifically bind to RNA polymerase and transcription factors to determine the initiation of gene transcription, and they contain many important *cis*-acting elements. Promoters receive signals from a variety of sources (such as cell receptors) and control the level of transcription initiation, which determines gene expression to a great extent and plays an important role in plant gene expression regulation [1,2]. Different promoters contain different regulatory elements. The analysis of relevant regulatory elements has become particularly important to help promote the understanding of gene expression patterns and further improve plant performance under unfavorable conditions.

Translationally controlled tumor protein (TCTP), a multifunctional protein, is highly expressed and ubiquitously distributed in all eukaryotes [3]. TCTP levels are regulated transcriptionally and posttranscriptionally [4-6]. In plants, TCTP plays a key role in stress response and signal transduction [7-10]. In rubber tree (Hevea brasiliensis), HbTCTP is regulated by drought, temperature, salt treatment, ethylene treatment, treatment, and wounding, H_2O_2 methyl jasmonate treatment [11]. In the developing seed of soybean (Glycine max), GmTCTP proteins interact with GmCDPKSK5 proteins and may function in high temperature and humidity stress responses [12]. Cucumber (Cucumis sativus) has two TCTPs. In 2014, CsTCTP1 (XP-004134215) was first found to play a vital role in the response of cucumber to Sphaerotheca fuliginea at the protein level through two-dimensional gel

analysis [13]. In our subsequent study, the CsTCTP1 and CsTCTP2 genes were both found to be negative modulators in the cucumber defense response to S. fuliginea. CsTCTP1 participates in the defense response to S. fuliginea through regulating the expression of defense-associated certain genes and/or pathwayabscisic acid (ABA) signaling associated genes, and CsTCTP2 participates by regulating the expression of target of rapamycin (TOR) signaling pathway-associated genes [14].

Although CsTCTP genes are induced by S. fuliginea, little is known about the function of CsTCTP promoters or CsTCTP expression regulation. In this study, the promoters of CsTCTP1 and CsTCTP2 were isolated and analyzed. Histochemical and fluorometric GUS assays were used to test their active sites. In addition, promoter activity was analyzed under treatments with different exogenous hormones (ABA, salicylic acid [SA], and ethylene [ETH]). This study will help to further understand the expression patterns and the regulatory mechanism of gene transcriptional regulation.

2. Materials and Methods

2.1 Plant Material and Treatments

Cucumber seeds (B21-a-2-1-2) with high resistance to *S. fuliginea* were obtained from the Liaoning Academy of Agricultural Sciences. The seeds were sterilized with 50% NaClO for 15 min and 75% ethyl alcohol for 45 s, then washed with ddH₂O 4 or 5 times. The treated seeds were germinated on solid Murashige and Skoog medium in a greenhouse under a light intensity of 40 lx at 24 °C. When grown to two true leaves, the seeds were carefully transferred into soil matrix and maintained in a growth chamber with a photoperiod of 16 h light, 8 h dark.

2.2 Isolation of CsTCTP1 and CsTCTP2 Promoters, pT1 and pT2

Total genomic DNA was isolated from the cucumber leaves using a genomic DNA kit. Using the genomic DNA as a template, the primer pairs pT1-1-Sall-F/pT1-Ncol-R (-1,308/+88, positions relative to the transcriptional start site [TSS]) and pT2-1-Sall-F/pT2-Ncol-R (-1,305/+69) were used to clone the promoter (Supplementary Table 1; underlining represents restriction sites).

Amplification reactions were carried out as follows: 94°C for 5 min, followed by 38 cycles of amplification (94 °C for 30 s, 60–65 °C for 30 s, 72°C for 2 min 15 s), and then 72°C for 10 min. Polymerase chain reaction (PCR) products were purified using a DNA gel extraction kit.

2.3 Bioinformatics Analyses

TSSs and possible core promoter regions were predicted using BDGP by the (http://www.fruitfly.org/seq_tools/promoter.html) TSSP and (http://linux1.softberry.com/berry.phtml?topic=tss p&group=programs&subgroup=promoter) tools. The *cis*-acting elements, distributions, and biological functions of CsTCTP1 and CsTCTP2 promoter sequences were analyzed by using the **PlantCARE** tool

(http://bioinformatics.psb.ugent.be/webtools/plant care/html/).

2.4 Plasmid Construction

The upstream regions of CsTCTP1 (-1,308 to +88) and CsTCTP2 (-1,305 to +69) were divided into three fragments by size (1.3, 0.7, and 0.3 kb). Different CsTCTP1 and CsTCTP2 promoter fragments were amplified by PCR with specific forward primers (pT1-2-SacI-F [-714/+88], pT1-3-Sall-F [-306/+88], pT2-2-Sacl-F [-732/+69], pT2-3-Sall-F [-324/+69]) and common reverse (pT1-Ncol-R, pT2-Ncol-R) primers (Supplementary Table 1). PCR products were digested with restriction enzymes and subcloned pCAMBIA1301 vectors, replacing the into CaMV35S promoters (previously eliminated by corresponding restriction enzyme digestion) to generate six CsTCTP_{pro}::GUS constructs (Fig. 1).

2.5 Agrobacterium-Mediated Transient Expression

Agrobacterium tumefaciens GV3101 cells harboring the $35S_{pro}$::GUS or CsTCTP_{pro}::GUS vector were cultured to OD600=0.5 in infiltration medium (100 mM MgCl₂, 200 µM acetosyringone, 20 mM MES, pH 5.6), then diluted to OD600=0.5. The diluted culture was injected into 5-week-old tobacco leaves (*Nicotiana benthamiana*) using a syringe without a needle [15]. *A. tumefaciens* GV3101 cells were also transformed into tobacco plants as negative controls. Three replicates within each independent experiment and three independent biological replicates were performed.

2.6 Histochemical and Fluorometric GUS Assays

The tobacco plants after injection were routinely grown in a culture chamber (25°C, 16 h light, 8 h dark) and sprayed with exogenous hormones (100 µM ABA, 1 mM SA, or 10 µL/L ETH) after 24 hours of treatment. The control group was treated with H₂O and collected after 24 h of continuous cultivation, and infiltrated leaf discs were used for histochemical GUS staining according to the method of Jefferson [16]. The plant samples were immersed in GUS staining solution (1 mg/mL X-Gluc, 100 mM NaH₂PO₄, 100 mM Na₂HPO₄, 5 mM K₃Fe(CN)₆, 5 mM K₄Fe(CN)₆, 1% Triton X-100, 1 mM EDTA, pH 7) for 16 h at 37°C, then washed with 70% absolute ethanol to remove chlorophyll until the negative control was colorless.

β-Glucuronidase activity was quantified by using fluorometric GUS assays. About 100 mg of tobacco leaves were frozen and milled in liquid nitrogen, and the total protein was extracted by resuspending the samples in 1 mL of extraction buffer (50 mM NaPO₄, pH 7, 10 mM EDTA, 10 mM β-mercaptoethanol, 0.1% sodium lauryl sarcosine, 0.1% Triton X-100). The homogenate was centrifuged at 12,000 rpm for 10 min, then supernatant recovered. the was The concentration of total protein was determined according to the method of Bradford [17] using bovine serum albumin as a standard. GUS activity was measured as described by Jefferson [16], and fluorescence of 4-methyl umbelliferone was recorded using a spectrofluorimeter with excitation at 365 nm and emission at 455 nm and expressed as nanomoles of 4-methyl umbelliferone generated per µg of protein per min.

3. RESULTS AND ANALYSIS

3.1 Isolation and Structural Analysis of *CsTCTP1* and *CsTCTP2* Promoters

The upstream 1400-bp fragment of the ATG of the *CsTCTP1* and *CsTCTP2* genes and its short

segments were successfully cloned by reverse transcription PCR. The PCR amplification products of the *CsTCTP1* and *CsTCTP2* promoters were detected by 1% agarose gel electrophoresis, and the results showed that these specific fragments were the same size as expected (Fig. 2).

Using the BDGP, PlantCARE, and PLACE tools, the structures of the *CsTCTP* promoters were predicted and analyzed. The results showed that the core promoter region of *CsTCTP1* was located at -129 to -79 bp, the TSS was C, which was located at -88 bp, and the TATA box was located at -28 bp upstream of the TSS (Fig. 3). The core promoter region of *CsTCTP2* was located at -109 to -59 bp, the TSS was A, which was located at -68 bp, and the TATA-box

was located at -28 bp upstream of the TSS (Fig. 4).

The *CsTCTP1* promoter contained three ABRE elements and one TCA element, which are involved in ABA and SA responsiveness, respectively. The *CsTCTP1* promoter also had some defense response elements, such as TC-rich repeats (involved in defense and stress responsiveness), HSE (involved in heat stress responsiveness), and MBS (MYB binding site involved in drought-inducibility) (Supplementary Table 2). The *CsTCTP2* promoter had ABRE and ERE elements, which are involved in ABA and ethylene responsiveness, respectively. Four TC-rich repeats (involved in defense and stress responsiveness) were found in the *CsTCTP2* promoter (Supplementary Table 3).

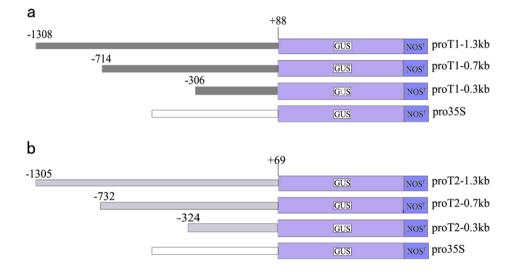


Fig. 1 Construction of *CsTCTP*_{pro}::*GUS*. Three differently sized *CsTCTP* promoter regions (0.3 kb_{pro}, 0.7 kb_{pro}, 1.3 kb_{pro}) were fused to the *GUS* reporter gene. NOS^T, *Agrobacterium tumefaciens NOS* gene transcription terminator

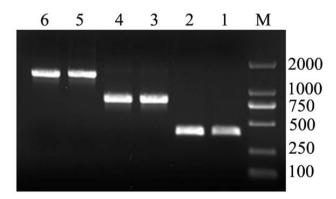


Fig. 2. Electrophoresis profile of the PCR products of the *CsTCTP1* and *CsTCTP2* promoters and their short segments. M: DL2000 DNA marker; 1,3,5: *CsTCTP1pro*; 2,4,6: *CsTCTP2pro*

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		TATA-	ATTITTAAAGATAA	G-BOX TATA-b			
-1360	αςττςτςταλαιτςςαταττολατς aggotttaatς αλας τατα <u>ταλτα</u> λοστος ατοτταστς αττος ΤΑΤΑ-box					TCATTGA	
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	AATGTTAAA CAAT-box	G-Box	TATTAC TACC ATTTA	ICGATACGTTAGCAAA	ATACTGAGTO	GAAGGA	
-1150	gagtgatgtactgataattaaaagaaagtgatttaattgatagtctaaataggtt <mark>gaati</mark> atti <u>katti</u> atti CAAT-box TATA- b						
1080	TTTATCTAA	TTATGTACATCTT	TGTTCAATCCGTAC CAAT-box	ACTATOTATAAAAATCAAC TATA-box	GTAACGTTC	AAAAGTT.	
-1010	TGAAACATA	TATA-box	CAAT-box AL	OTO AATAGGATATTCAAA	ATAAAAGCA	CACTTTA	
-940	CATTTAAAA TATA-bo			ACTITITATACTOTATAAA TATA-box	CAATTITAA		
-\$70	CCAGTGTG	AATAACAAAAAT	TATTCTATCGATTAA	CCACTCTTACATGTATGA.	AAAATTTGAT	ACACGAT	
-800	CATTCAGATCTTOGTACATGATCTTOTACCAATTGTTAACAATCATGCATGATAATAATTTTTGGTACACGATC. CAAT-box CAAT-box						
			CAAT-box	CAAT-box			
-730	GTTTAGATT	TAGTACAAGAGC		CAAT-box	CGTGTACTC	AAGATCT	
			GTATAGCAAAATCT.	CAAT-box			
-660	TCTATATTT	ggtatactattgt	GTATAGCAAAATCT. TTAGACTTATAAGA	CAAT-box	AATGAATATT	GTTTACC.	
-660 -590	TCTATATTT AGTTGGAA	GOTATAC TATTOT ATAT GAA GAA GTA GAA GAATT TATA G	GTATAGCAAAAATCT. TTAGACTTATAAGA AAAAAATGAAGAAA	CĀAT-box Αλακτοαττιττόττς αόα αλαλατ <u>άτατλα</u> οα όατι ΤΑΤΑ-box Αλακτατοτοία <u>ς ας όα ό</u> ας <u>G-box</u> Ολατατοδαλαλαλαλαλα	AATGAATATT AGAAGAAAAT TA	otttacc. Tatagaa TA-box	
-730 -660 -590 -520 -450	тстататтт асттосаал саалалас	GOTATACTATTOT ATATGAAGAAGTA/ GAAGAATTTATAG C AATTTTATATTTT	отатаосалаатст ттаоасттаталоа алалалатоалодала ж <u>салт</u> алсолотоа :ААТ-Бох	CĀAT-box Αλακτοαττιττόττς αόα αλαλατ <u>άτατλα</u> οα όατι ΤΑΤΑ-box Αλακτατοτοία <u>ς ας όα ό</u> ας <u>G-box</u> Ολατατοδαλαλαλαλαλα	ATGAATATT AGAAGAAAAT TA TA TAATAATA IATA-box CATTTATQ C	OTTTACC. TATADAA. TA-box (AAAAAA) HSE	
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-660 -590 -520 -450	тстататтт аоттосаал салаалас (ттта)ттаал сс(таата)ст ТАТА-box	оотатастаттот ататоалодаотал одалодатттатдо с алттттататттт гл одатаод <u>гаата</u> тта GA-motif <u>тат</u>	οταταος αλαλάτος τταοας τταταλοβά αλαλαλάτολαολολ (ΑΓΑΔ) μας ο αλαγγραφικός ΑΓΑΔ) μας στη τη τ	СААТ-box маатоаттитиотисаол алалат <u>битатал</u> ололг. ТАТА-box олагатоалалалалалал отатиталта <u>бити</u> датобалаодаоболоо	AATGAATATT AGAAGAAAAT TA TAATAATATG IATA-box CATTATATG CAU AATAATGAAA	GTTTACC. TATADAA. TA-box HSE AAATI TA. AT-box NTTAGCAT.	
-660 -590 -520 -450 -380	тстататтт аоттооааа балалалас Тттаттала сс <u>талта</u> ст тата-box таласатта	оотатастаттот ататоалоалотал оалоалттатао с алатттататттт с с алаос <u>палат</u> ят. с GA-motif тат асталасталало	отатаос алаатет. ттабаеттаталора алаалааторалорала Сал аралоралора Сал аратора Сал араторатора		AATGAATATT AGAAGAAAAT TA TA TAATAATATG IATA-box CATTTATG CA AATAATGAAA CATACATATAT X	OTTTACC. TATADAA. TA-box HSE AAATI TA. AT-box XTTAGCAT.	
-660 -590 -520 -450 -380 -310	тстататтт абттобаал оллаалас <u>ттта</u> ттаал сс <u>таата</u> ст тата-box таалсатта атараттаал тата-box	оотатастаттот алагоалолатттатао салоалттатао с алтттататтті с с с с с с с с с с с с с с с с с с	οτατάος αλαλάτος τταοάς τταταλοά Αλαλαλατολάολα Αλαλάλας ο Αλά Αλατικός Αλατικός Αλατικός Αλατικός Αλαστικός Αλαστικός Αλαστικός Αλατικός Αλαστικός Αλατικός Αλατικός Αλατικός Αλατικός Αλαλολαζο Αλαλολαλοδο Αλαλολολαζο Αλαλολαλολαζο Αλαλολαζο Αλαλολαλολαζο Αλαλολολαζο Αλαλολολαζο Αλαλολολαζο Αλαλολολαζο Αλαλολολαζο Αλαλολολολαζο Αλαλολολαζο Αλαλολολαζο Αλαλολολολολολολολολολολολολολολολολολολ		AATGAATATT TA TA TATATATA FOR TATA-box FATA-box CA AATAATGEAA X TAACATATA X X TAACATATGCAA X X X AATCGGAATT	OTTTACC. TA-DOX TA-DOX MAAAAA HSE AAAT] TA- AT-DOX AT-TACAT AT-ACAT AT-ACAT	
-660 -590 -520 -450 -380 -310 -240	тстататтт афтгодаля оалаалас (1112)гталя сс <u>блат</u> ест ТАТА-box Таласаття ТАТА-box таластат		отатаосалалатст ттаоасттаталол лалалатоллолал лалалатоллолал лалаласалатоста ала татостасс алалалата лалоза салоста сал	СААТ-box АЛАТСАЛТТІТОТІСАОА ОЛАЛАТ <u>СТАТАЛ</u> ОЛОЛІ ТАТА-box АЛАТАТОТОА-САОХСОЛ С-box ОЛАТАТОЛО-С-бох ОЛАТОЛО-С-бох ОЛАТАТОЛО-С-бох ОЛАТАТОЛО-С-бох ОЛАТОЛО-С-БОХ ОЛАТОЛО-С-БОХ ОЛАТОЛО-С-БОХ	ΑΑΤΟΑΑΤΑΤΤ ΑΟΛΑΟΑΑΔΑ ΤΑΙ ΤΑΙ ΤΑΑΤΑΑΤΟ ΓΑΤΑ-Βοχ CATITATOς CATITATOς ΑΑΤΑΑΙΟΑΑΑΤ ΑΑΤΑΟΑΤΟΡΟΙ ΙΙΑ-Βοχ	отттасс. Талара - Талара Талара НЗЕ Алат] Та. АТ-Бох хттассат. тасст <u>атт]</u> - адаада. Таосстс. стттоса.	

Fig. 3 Sequence and structural analysis of the *CsTCTP1* promoter. Red underline: the core region of the promoter; arrow: transcription start site; black underline: start codon; box: *cis*-regulatory element

-1430	ATTTATOATCAATOGAOTAATTATTTOTAACCATTTTTOTOTTOTATTOTTOTTOTATTOTTOTAOATOAOTT <u>CAA</u> - TATA-box CAAT-box CAAT-box	x
-1360	ПАСАТОС АОСССАТАО <mark>НОЛАХСАХ</mark> ОЛАОТСАЛАЛОТТАООСТССАЛАЛТАТОСТТІСТТОСТТАОЛАЛО. AE-box	
-1290	GAATGCTITGTTTTGTAAGATTAGTAGAGAGAGAGAGAGAGAGAGA	
-1220	1CAATIAAACAAATIAAGAAACCTTTTTTTTTTTTTTTTTT	
-1150		
-1080	ΤΟΘΟΤΟΤΑΤΤΑΘΑΑ <mark>ΔΤΑΤΑΛΤΑ</mark> ΑΤΤΑΘΑΑCΤΑ <mark>ΕСΑΛΤΙ</mark> CΑΑΛΑΤΑΑΑCΑΑΑ <u>C</u> ΓΑΑΤΗΓΑΛΟΤΙΑΛΟΤΙΑΛΟΤΙΑΛΟΤΙΑΛΟΤΙΑΛΟΤΙΑΛΟΤΙΑΛΟΤΙ	
-1010 T.4	[ΤΩΤΤΟΟ <u>ΕΛΑΛΙ</u> ΝΤΤΟΟΤΑΑΑΑΟΑΑΑΤCATI <u>ΓΙΑΤΑ</u> ΙΤΟΑΤ <u>ΕΛΑΤΙΈΛΑΑΤ</u> ΟΑΑΟΑΑΟCATTAACATOAOAT. IA-box CAAT-box CAAT-box CAAT-box CAAT-box	
-940	AAAAT ITAATAAAAAAAAAAAAAAAAAAAAAAAAAAAA	
-\$70	AATCOMACCOMACAACTAAGAACAACAACTACAAATINATITITTADOTTCTTTTCTAAGAACCAATING AAAC-motif CAAT-box TATA-box ELI-box3 CAAT-box	
-800 TC-	-ATTENDATION CACCTCACTTCTTI <u>FITTAAAAA</u> AAATAGTAATTTTTC <u>TATACAFATA</u> CA <u>FATA</u> CA	
-730	$\begin{tabular}{lllllllllllllllllllllllllllllllllll$	
-660	AGT <u>EAAT</u> BATTI <u>TITIA</u> ITECACTITEATECTITIATAGAGACAGAATATGECTATATGEATTGATTET. CAAT-box TATA-box	
-590	CATCTTOGCAGCCAC <mark>CAAATH</mark> AATATATATATATATATATATATATATTCATTTCOTATCITTAAGAACGA. CAAT-box TATA-box TATA-box	
-520	agaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaaa	
-450	αυτοαστοατιτοταλαλαφ <u>ελατι</u> αλουτιστι <u>τιτιτα</u> ολαγτιτισ <u>ιτιταλαλαλ</u> ατιατολασατο <u>γγαλαλα</u> CAAT-box TATA-box TATA-box TATA-box TATA-box	
-380	CACTTACTITC <u>TAATA</u> CACOTO <u>TTTTA</u> AAGOTATAATTTTCTTTCTTOTOAATTATTT <mark>OTTTTCTTAA</mark> AA. TATA-box TATA-box TC-rich repeats	
-310	αλααττομ <u>ατιατοματι</u> ολααλας αγταατιαλιταιόταλαος αλααλογιάλασταλς οταλαγ <u>τιταλα</u> ΤΑΤΑ-box ΤΑΤΑ-box	
-240	масслаталалот <mark>слалода</mark> с алалттатта <mark>те тта</mark> лаласалалаладалаттадалалталосотосло. AAGAA-motif TATA-box	
-170	GGTAATTTGGTAATTAGAGAAAATCGTAAATGAGAATCCTAATCGTATTCGGATTTGAATTG <mark>AATTGTGG.</mark>	
-100	TATAAATAGGAGAGAGCTTATAGCOTTCTTTACCGCAAATCTTCCTTCTTCTTTGAATTGOTTCTTCAT TATA-box CAAT-box TC-rich repeats	
-30	ATTTT <u>CAATT</u> CTCTTTTCCAGCCACAGCC <u>ATOC</u> TTCTCTACCAAGACCTTCTAACAG . CAAT-box	

Fig. 4. Sequence and structural analysis of the *CsTCTP2* promoter. Red underline: the core region of the promoter; arrow: transcription start site; black underline: start codon; box: *cis*-regulatory element

3.2 Analysis of *CsTCTP1* and *CsTCTP2* Promoter Activity in Transgenic Tobacco

In our previous study, we found that there were no differences in the sequences of the promoters between two sister cucumber lines, B21-a-2-2-2 and B21-a-2-1-2 [18]. In this study, we chose cucumber line B21-a-2-1-2 as the material for sequence isolation. Based on the results of the analysis of the Arabidopsis TCTP promoter [19] and the structural analysis of the CsTCTP promoters, we generated transgenic plants with three short segments of CsTCTP promoters that were fused to GUS, and we then determined the activity of the promoters.

With transgenic tobacco the plants of CsTCTPpro::GUS vectors, histochemical and fluorometric GUS assays were conducted to analyze promoter activity in the plants (Fig. 5). The results showed that the 0.7-kb CsTCTP1 promoter (proT1-0.7kb) and 0.7-kb and 1.3-kb CsTCTP2 promoters (proT2-0.7kb and proT2-1.3kb) had strong GUS expression, which was comparable to that of the control plant with the 35S promoter (35Spro::GUS). The proT1-0.7kb promoter showed the strongest GUS activity compared to proT1-0.3kb and proT1-1.3kb, corresponding to approximately five-fold GUS activity of $35S_{pro}$::GUS. The proT2-0.7kb and proT2-1.3kb promoters showed the strongest GUS activity compared to proT2-0.3kb, corresponding to approximately three-fold GUS activity of 35Spro::GUS.

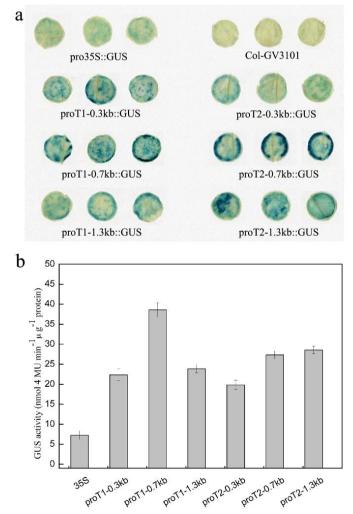


Fig. 5. Promoter activity analyses with upstream regions of *CsTCTP1* and *CsTCTP2*. a. Histochemical GUS analysis. b. Fluorometric GUS analysis. Each measurement was repeated three times. GV3101 and *pro35S::GUS* leaf discs were used as negative and positive controls, respectively

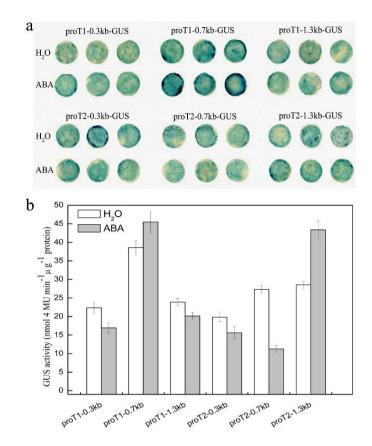


Fig. 6. GUS staining and fluorescence quantitative assay after ABA treatment. The H₂O-treated group was used as the control

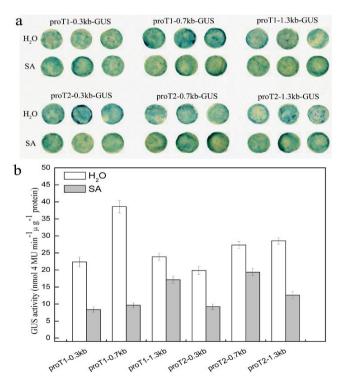


Fig. 7. GUS staining and fluorescence quantitative assay after SA treatment

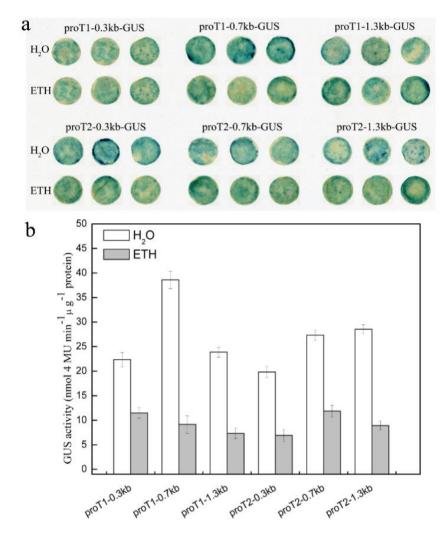


Fig. 8. GUS staining and fluorescence quantitative assay after ETH treatment

3.3 Effects of Exogenous Hormones on *CsTCTP1* and *CsTCTP2* Promoter Activity

CsTCTP1 and CsTCTP2 are regulated by exogenous hormone treatments [18]. Furthermore, their promoters contain several *cis*-elements associated with ABA, SA, ETH. То explore effects and the of hormones exogenous CsTCTP1 on and CsTCTP2 promoter activity, the transgenic tobacco leaves were divided into three groups and sprayed with 100 µM ABA, 1 mM SA, or 10 µL/L ETH. After 24 h of treatment, each group was tested by histochemical and fluorometric GUS assays.

The GUS activity of the proT1-0.7kb and proT2-1.3kb promoters was remarkably enhanced under ABA treatment compared to under H_2O treatment (Fig. 6). Further, the GUS activity of proT2-0.7kb was reduced, but it did not change significantly for the other promoters (proT1-0.3kb, proT1-1.3kb, proT2-0.3kb). When under SA or ETH treatment, the GUS activity of all the promoters was reduced to varying extents compared to under H_2O treatment (Fig. 7 and 8). These results are supported by the previous bioinformatics analysis, which revealed SA and ETH response elements in the promoters of *CsTCTP1* and *CsTCTP2*, and it can be speculated that SA and ETH may negatively regulate the promoters of *CsTCTP1* and *CsTCTP2*.

4. DISCUSSION

TCTP is a highly conserved protein that exists in eukaryotes [3]. TCTP is considered to play an essential role in the regulation of growth and development in eukaryotes [20-23]. However, gene expression regulation by upstream promoters is not understood. In this study, the reporter gene used was an intron containing the GUS gene (Fig. 5), which ensured that the expression was plant cell-specific. The results showed that all of the six promoter deletion fragments of CsTCTP1 and CsTCTP2 had the function of initiating gene expression. The transcription activity of proT1-0.7kb in tobacco corresponded to about five-fold activity compared with 35Spro::GUS, which showed that proT1-0.7kb (-720 bp to -1313 bp) contained a cisacting element similar to the enhancer. It was reported that the 0.3-kb AtTCTP promoter in Arabidopsis exhibited the strongest transcription activity, while the 0.7-kb CsTCTP1 promoter and 0.7-kb and 1.3-kb CsTCTP2 promoters in this study exhibited the strongest transcription activity (Fig. 5). One explanation for these results is that: (a) the CsTCTP1 promoter shares only 41.37% nucleotide identity with the Arabidopsis AtTCTP promoter, while the CsTCTP2 promoter shares only 38.25% nucleotide identity with the AtTCTP promoter, and (b) transient transformation is different from stable transformation. Indeed, transient expression has some deficiencies that limit its application. The results of this experiment still need to be further validated by the combination of stable expression analysis. However, transient expression can be a quick and preliminary way to understand the functional properties of CsTCTP promoters. Furthermore, the tobacco system is more mature and accepted.

In plants, SA is an important defense signaling molecule. In this study, SA may have had a negative regulatory effect on the CsTCTP1 and CsTCTP2 promoters. This work proved once again that CsTCTP1 and CsTCTP2 are both negative modulators in the cucumber defense response to S. fuliginea. Despite showing the same negative effects on S. fuliginea, CsTCTP1 slightly and CsTCTP2 displayed different expression patterns. The 1.396-bp CsTCTP1 promoter and 1.374-bp CsTCTP2 promoter sequences (relative to ATG) showed only 44.09% nucleotide identity. This low sequence homology can be explained by the different regulatory mechanisms of CsTCTPs. Compared with H₂O-treated tobacco, the ABA-treated group showed that the activity of proT1-0.7kb and proT2-1.3kb Combined increased. with bioinformatics predictions, this may be explained by the presence of defensive stress response elements in their promoter sequences. The GUS activity of proT2-0.7kb was reduced, probably due to the presence of negative regulators of ABA within the range of -324 bp to -732 bp.

However, the activity of proT1-0.3kb, proT1-1.3kb, and proT2-0.3kb did not change significantly, so it is speculated that no relevant hormone response elements existed in these fragments.

Promoter prediction is still a challenging task, although different methods have been proposed for prediction, but for new resistance-related promoter clones, *cis*-acting elements have been identified by specific sequences between the elements. The transcription factors that interact with these elements will be the focus of future promoter studies.

5. CONCLUSION

In summary, the 0.7-kb *CsTCTP1* promoter (proT1-0.7kb) and 0.7-kb and 1.3-kb *CsTCTP2* promoters (proT2-0.7kb and proT2-1.3kb) had strong transcriptional activity. The proT1-0.7kb promoter of *CsTCTP1* and proT2-1.3kb promoter of *CsTCTP2* may contain ABA-related response elements, and SA and ETH may have negative regulatory effects on the *CsTCTP1* and *CsTCTP2* promoters.

SUPPLEMENTARY

Supplementary table is available in this link: https://www.journalbji.com/index.php/BJI/libraryFi les/downloadPublic/13

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

- 1. Dieterich C, Grossmann S, Tanzer A, Röpcke S, Arndt PF, Stadler PF, Vingron M. Comparative promoter region analysis powered by CORG. BMC Genomics. 2005;6:24.
- 2. Nie LN, Xia LQ, Zhao-Shi XU, Gao DY, Lin LI, Zhuo YU, Chen M, Lian-Cheng LI, You-Zhi MA. Progress on cloning and functional

study of plant gene promoters. J Plant Genet Resour. 2008;9(3):385-391.

- 3. Bommer UA, Thiele BJ. The translationally controlled tumour protein (TCTP). Int J Biochem Cell Biol. 2004;36(3):379-385.
- MacDonald SM, Bhisutthibhan J, Shapiro TA, Rogerson SJ, Taylor TE, Tembo M, Langdon JM, Meshnick SR. Immune mimicry in malaria: Plasmodium falciparum secretes a functional histamine-releasing factor homolog in vitro and in vivo. PNAS. 2001;98(19):10829-10832.
- Arcuri F, Papa S, Meini A, Carducci A, Romagnoli R, Bianchi L, Riparbelli MG, Sanchez JC, Palmi M, Tosi P, Cintorino M. The translationally controlled tumor protein is a novel calcium binding protein of the human placenta and regulates calcium handling in trophoblast cells1. Biol Reprod. 2005;73(4):745-751.
- Hsu YC, Chern JJ, Cai Y, Liu M, Choi KW. Drosophila TCTP is essential for growth and proliferation through regulation of dRheb GTPase. Nature. 2007;445(7129):785-788.
- Shen QH, Saijo Y, Mauch S, Biskup C, Bieri S, Keller B, Seki H, Ulker B, Somssich IE, Schulze-Lefert P. Nuclear activity of MLA immune receptors links isolate-specific and basal diseaseresistance responses. Science. 2007;315(5815):1098-1103.
- Kim YM, Han YJ, Hwang OJ, Lee SS, Shin AY, Kim SY, Kim JI. Overexpression of Arabidopsis translationally controlled tumor protein gene AtTCTP enhances drought tolerance with rapid ABA-induced stomatal closure. Mol Cells. 2012;33(6):617-626.
- 9. Rosenberger CL, Chen J. To grow or not to grow: TOR and SnRK2 coordinate growth and stress response in Arabidopsis. Mol Cell. 2018;69(1):3-4.
- 10. Branco R, Masle J. Systemic signalling through translationally controlled tumour protein controls lateral root formation in Arabidopsis. J Exp Bot. 2019;70(15):3927-3940.
- 11. Deng Z, Chen J, Leclercq J, Zhou Z, Liu C, Liu H, Yang H, Montoro P, Xia Z, Li D. Expression profiles, characterization and function of HbTCTP in rubber tree (*Hevea brasiliensis*). Front Plant Sci. 2016;7:789.
- 12. Wang S, Tao Y, Zhou Y, Niu J, Shu Y, Yu X, Liu S, Chen M, Gu W, Ma H. Translationally controlled tumor protein GmTCTP interacts with GmCDPKSK5 in response to high temperature and humidity

stress during soybean seed development. Plant Growth Regul. 2017;82(1):187-200.

- Fan H, Ren L, Meng X, Song T, Meng K, Yu Y. Proteome-level investigation of Cucumis sativus-derived resistance to *Sphaerotheca fuliginea*. Acta physiol Plant. 2014;36(7):1781-1791.
- 14. Meng X, Yu Y, Zhao J, Cui N, Song T, Yang Y, Fan H. The two translationally controlled tumor protein genes, CsTCTP1 and CsTCTP2, are negative modulators in the Cucumis sativus defense response to *Sphaerotheca fuliginea*. Front Plant Sci. 2018;9:544.
- Yang Y, Li R, Qi M. In vivo analysis of plant promoters and transcription factors by agroinfiltration of tobacco leaves. Plant J. 2000;22(6):543-551.
- Jefferson RA. Assaying chimeric genes in plants: the GUS gene fusion system. Plant Mol Biol Rep. 1987;5(4):387-405.
- 17. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of proteindye binding. Anal Biochem. 1976;72:248-254.
- Meng XN, Chen QM, Fan HY, Song TF, Cui N, Zhao JY, Jia SM, Meng KX. Molecular characterization, expression analysis and heterologous expression of two translationally controlled tumor protein genes from Cucumis sativus. PLoS One. 2017;12(9):e0184872.
- Han YJ, Kim YM, Hwang OJ, Kim JI. Characterization of a small constitutive promoter from Arabidopsis translationally controlled tumor protein (AtTCTP) gene for plant transformation. Plant Cell Rep. 2015;34(2):265-275.
- 20. Gachet Y, Tournier S, Lee M, Lazaris-Karatzas A, Poulton T, Bommer UA. The growth-related, translationally controlled protein p23 has properties of a tubulin binding protein and associates transiently with microtubules during the cell cycle. J Cell Sci. 1999;112 (Pt 8):1257-1271.
- 21. Li F, Zhang D, Fujise K. Characterization of fortilin, a novel antiapoptotic protein. J Biol Chem. 2001;276(50):47542-47549.
- 22. Lucibello M, Gambacurta A, Zonfrillo M, Pierimarchi P, Serafino A, Rasi G, Rubartelli A, Garaci E. TCTP is a critical survival factor that protects cancer cells from oxidative stress-induced celldeath. Exp Cell Res. 2011;317(17):2479-2489.

23. Shen JH, Qu CB, Chu HK, Cui MY, Wang YL, Sun YX, Song YD, Li G, Shi FJ. siRNA targeting TCTP suppresses osteosarcoma

cell growth and induces apoptosis in vitro and in vivo. Biotechnol Appl Biochem. 2016;63(1):5-14.

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