



Heterotrimeric G-Protein γ Subunit CsGG3.2 Positively Regulates the Expression of *CBF* Genes and Chilling Tolerance in Cucumber

Longqiang Bai¹, Yumei Liu^{1,2}, Ying Mu¹, Ali Anwar¹, Chaoxing He¹, Yan Yan¹, Yansu Li^{1*} and Xianchang Yu^{1*}

¹ Institute of Vegetables and Flowers, Chinese Academy of Agricultural Sciences, Beijing, China, ² College of Agricultural and Biological Engineering, Heze University, Heze, China

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*Correspondence:

Yansu Li liyansu@caas.cn; liyitian816@163.com Xianchang Yu xcyu1962@163.com

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Bai L, Liu Y, Mu Y, Anwar A, He C, Yan Y, Li Y and Yu X (2018) Heterotrimeric G-Protein γ Subunit CsGG3.2 Positively Regulates the Expression of CBF Genes and Chilling Tolerance in Cucumber. Front. Plant Sci. 9:488. doi: 10.3389/fpls.2018.00488 Heterotrimeric guanine nucleotide-binding proteins (G proteins) composed of alpha (G α), beta (G β), and gamma (G γ) subunits are central signal transducers mediating the cellular response to multiple stimuli, such as cold, in eukaryotes. Plant Gy subunits, divided into A, B, and C three structurally distinct types, provide proper cellular localization and functional specificity to the heterotrimer complex. Here, we demonstrate that a type C Gy subunit CsGG3.2 is involved in the regulation of the CBF regulon and plant tolerance to cold stresses in cucumber (Cucumis sativus L.). We showed that CsGG3.2 transcript abundance was positively induced by cold treatments. Transgenic cucumber plants (T1) constitutively over-expressing CsGG3.2 exhibits tolerance to chilling conditions and increased expression of CBF genes and their regulon. Antioxidative enzymes, i.e., superoxide dismutase, catalase, peroxidase, and glutathione reductase activities increased in cold-stressed transgenic plants. The reactive oxygen species, oxygen free radical and H₂O₂, production, as well as membrane lipid peroxidation (MDA) production decreased in transgenic plants, suggesting a better antioxidant system to cope the oxidative-damages caused by cold stress. These findings provide evidence for a critical role of CsGG3.2 in mediating cold signal transduction in plant cells.

Keywords: G-protein γ subunit, CsGG3, cucumber, cold stress, CBF genes

INTRODUCTION

Cold stress, which includes chilling ($<20^{\circ}$ C) and/or freezing ($<0^{\circ}$ C) temperatures, adversely affects the growth and development of crops, and results in heavy economic losses (Chinnusamy et al., 2007). Plants exhibit an increase in chilling tolerance after being exposed to low non-freezing temperatures, which is known as cold acclimation (Medina et al., 2011). During cold acclimation, genes coding CBFs (C-repeat binding factors), HSFC1 (heat shock transcription factor C 1), ZAT12 (zinc transporter of *Arabidopsis thaliana* 12), and CZF1 (CCCH-type zinc finger 1) are induced, and in turn regulate downstream cold responsive gene (COR) expression making changes in metabolism and physiological processes (Jia et al., 2016; Zhao et al., 2016). These include the changes in the activity of antioxidant enzymes, such as ascorbate peroxidase (APX), superoxide dismutase (SOD), and catalase (CAT), which contributes to increased freezing tolerance (Streb et al., 2002; Janda et al., 2003; Zhang et al., 2008). CBFs, also known as dehydration-responsive

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	MASSLDEHLVTS	ASIGVGAV	VDSRGKHRILAELKRL	EQELRYDQDDDDEVDKMGNIS	SICKDLLPCIETKTDP	LLFVLNGVV.NPSWDF	RWFECSPSSPE
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Glyma10g32214; GmGγ5: Glycine max L. Glyma11g18050; GmGγ6: Glycine max L. Glyma14g17000; GmGγ7: Glycine max L. Glyma17g29590; GmGγ8: Glyc max L. Glyma15g19631; GmGγ9: Glycine max L. Glyma17g05640; GmGγ10: Glycine max L. Glyma.07G040200; SIGGA1: Solanum lycopersicum Solyc09g082940.2.1 [Tomato Genome (cv Heinz; ITAG release 2.40) accession No.]; SIGGB1: Solanum lycopersicum Solyc12g096270.1.1; SIGGB2: Solanum lycopersicum Solyc08g005950.2.1; SIGGC1: Solanum lycopersicum Solyc07g041980.2.1.

element (DRE) binding factor 1 (DREB1), are key transcription factors involved in cold response (Jia et al., 2016). CBF genes are modulated by several transcriptional factors (TFs), including ICE1 (inducer of CBF expression 1), MYB15 (Myb domain protein 15), and CAMTA3 (calmodulin-binding transcription activator 3) (Jia et al., 2016; Zhao et al., 2016). Cold stress sensing leads to a cytosolic calcium (Ca²⁺) signal (Knight et al., 1996). The activated Ca²⁺ signal is postulated to activate a MAPK (mitogen-activated protein kinase) cascade, which phosphorylate TFs, such as CAMTAs and ICE1/2 (Zhu, 2016). Recently, Ma et al. (2015) reported that the interaction between heterotrimeric guanine nucleotide-binding protein (G protein) and CHILLING TOLERANCE DIVER GENCE 1 (COLD1) activates the Ca²⁺ signal upon cold treatment in rice. However, it is not well-established how G protein triggers Ca²⁺ signal.



FIGURE 2 Phylogenetic analysis of CsGGs and other $G\gamma$ proteins in plants. Tree was constructed using neighbor-joining method with 1000 bootstrap replicates.

G protein signaling pathway is an evolutionarily conserved extracellular signal transduction. G proteins comprises three distinct subunits: alpha (G α), beta (G β), and gamma (G γ) (Tuteja and Sopory, 2008). According to the canonical paradigm, ligandbound G protein coupled receptors (GPCRs) catalyzed exchange of GDP for GTP on the G α actives the heterotrimer, and resulting in dissociation of the two functional elements, Ga subunit and $G\beta\gamma$ dimer, which mediate signal transduction by interacting with multiple downstream effectors, independently (Yuri et al., 2012). In plants, G proteins and play significant roles in many stress responses. For instance, in rice and maize, Ga functions at both cell division and cellular senescence stages of plant responses to NaCl stress (Urano et al., 2014). Microarray analysis revealed that rice Ga plays an important role in the regulation of multiple abiotic stresses, such as drought, salinity, heat, and cold (Jangam et al., 2016). Arabidopsis GB (AGB1) positively regulates salt tolerance by affecting the expression of genes related to proline biosynthesis, oxidative stress, ion channel, and ABA-responses (Ya-Nan et al., 2015; Swain et al., 2016). It was also reported that transcripts of $PsG\alpha$ and $PsG\beta$ increased after heat, H₂O₂, and NaCl treatments in Pisum sativum, and overexpression of PsGa enhanced tolerance to salinity and heat in transgenic lines (Misra et al., 2007). However, the available set

of subunits in plants are limited. In Arabidopsis genome, there is only one G α gene (*GPA1*) and one G β gene (*AGB1*), while three G γ genes (*AGG1*, *AGG2*, and *AGG3*) (Daisuke et al., 2013). And this is roughly the G protein inventory for plants; for example, rice genome contains only one canonical G α gene (RGA1), one G β gene (RGB1), but five G γ subunits (RGG1, RGG2, GS3, DEP1, and OsGGC2) (Yuri et al., 2012; Daisuke et al., 2013). With single G α and G β subunits, the specificity of heterotrimer formation is thus solely provided by the G γ proteins (Trusov et al., 2007, 2008). It is thus important to study the roles of G γ subunits in triggering Ca²⁺ signaling for chilling tolerance in plant.

In cucumber, a typical chill-sensitive vegetable crop widely cultivated in the world, low temperatures can result in chilling injuries and lead to significant yield decreases. In the present study, we identified six G γ proteins encoded by the cucumber genome. The transcript levels of *CsGG3.2*, encoding a type C G γ , were up-regulated by cold treatment. *CsGG3.2* over-expressing enhanced tolerance of cucumber to chilling stress, and positively regulated the expression of *CBF* genes and their regulon, as well as activity of enzymes related to reactive oxygen species (ROS) scavenging. We conclude that the type C G γ subunit CsGG3.2 mediates cold signal transduction in cucumber.

MATERIALS AND METHODS

Plant Materials and Growth Conditions

Cucumber (*Cucumis sativus* L.) line 9930 donated by Huang et al. (2009) was used for gene cloning and "Xintai Mici" was used for the construction of transgenic plants. Cucumber seeds germinated in darkness at 28°C were sowed in vermiculite-peat mixture [1:1, volume/volume (V/V)] in the growth chamber under a 12 h light (350 μ mol m⁻² s⁻¹) at 25°C/12 h dark at 18°C cycle. Plants with two true leaves were exposed to cold stress at 8 \pm 1°C for 7 days, with 350 μ mol m⁻² s⁻¹ light for 12 h every day according to Wang et al. (1999).

Plant Transformation

To generate over-expression lines, the coding sequence of CsGG3.2 was amplified and cloned into the BamHI/SpeI sites of the pCAMBIA-2300 vector to obtain the Pro35S:CsGG3.2 construct. Agrobacterium LB4404 harboring the construct was used for cucumber transformation. The transformation was conducted according to the method of Mu et al. (2017). Briefly, cucumber seeds were rinsed in sterile deionized water for five times and placed on MS0 medium (MS plus 3% sucrose) for 2-3 days after being disinfected with 70% alcohol for 20 s followed by 3% sodium hypochlorite solution for 7 min. The basal cotyledons were then harvested and incubated with Agrobacterium harboring the target constructs for 15 min. The inoculated explants were then cultured on MS1 medium (MS0 medium plus 0.5 mg L^{-1} 6-Benzylaminopurine and 1 mg L^{-1} ABA) for another 2 days in the dark. The explants were transferred and incubated on MS1 medium for 15-20 days until the shoots were 1-1.5 cm long. The shoots were transferred to MS2 (MS plus 200 mg L^{-1} cefotaxime) to develop the roots.



Polymerase chain reaction (PCR) was employed to confirm the integration of the construct in regenerated plants.

Quantitative Real-Time Polymerase Chain Reaction (qRT-PCR)

Total RNA was isolated using RNA prep pure Plant Kit (TANGEN) and first-strand cDNA was synthesized using Fast Quant RT Kit (TANGEN) according to the manufacturer's instructions. PCR was then carried out using the gene-specific primers listed in Supplementary Table S1 and Super Real PreMix Plus (SYBR Green) Kit (TANGEN) with an Mx3000p Real-time PCR System (Agilent, Stratagene) according to the manufacturer's instructions. Three biological replicates were included for each sample. The $2^{-\Delta \Delta Ct}$ method was used, and the relative expression levels were normalized to *Actin*.

Biochemical Analysis Assays

Oxygen free radical (OFR), H2O2, and malondialdehyde (MDA) content, activity of SOD, catalase (CAT), peroxidase (POD), and glutathione reductase (GR) were determined using assay kits (COMINBIO) with a UV-1800 Spectrophotometer (SHIMADZU) according to the manufacturer's instructions. OFR content was assayed based on the detection of the absorbance of product at 530 nm in the reaction system. H_2O_2 content was assayed based on the titanium superoxide synthesis method. MDA content was assayed based on the thiobarbituric acid-reactive substance assay. The SOD activity was determined based on the inhibition of formazan synthesis method. The CAT activity was determined based on the decomposition of H2O2 method. The POD activity was assayed based on the detection of the absorbance of product at 470 nm in the reaction system. The GR activity was determined based on the NADPH consumption method.

Assessment of Chilling Tolerance of Cucumber

T1 transgenic lines and control plants with/without coldacclimated were exposed to $4 \pm 1^{\circ}$ C for 4 days, with 350 µmol m⁻² s⁻¹ light for 12 h every day. Chilling injury (CI) was indexed following Liu et al. (2010). The severity of the symptoms was assessed visually in a four-stage scale: (1) no injury; (2) slight; (3) moderate; (4) extensive. The average extent of cold damage was expressed as CI index, which was calculated using the following formula: CI index (%) = [Σ (CI level) × (number of seedlings at the CI level)/(total number of seedlings) × 4] × 100. Cold acclimation was performed at continuous 8 ± 1°C with 12 h photoperiod (350 µmol m⁻² s⁻¹) for 2 days.

Data Analyses

The results were analyzed using GraphPad Prism 6.0 (GraphPad Software) and Data Processing System (DPS) 7.05 (Tang and Zhang, 2013). Three biological replicates were included for each experiment. Data are presented as mean values \pm SE (n = 3). The analyses of significant differences (p < 0.05/p < 0.01) were measured using least significant difference (LSD) test.

RESULTS

The Cucumber Proteome Contains Six Heterotrimeric G Protein Gy Subunits

BLAST searches of the cucumber genome¹ using Arabidopsis $G\gamma$ subunits as queries identified five $G\gamma$ -like genes. We named these genes *CsGG1* (*Csa2G228360*), *CsGG2.1* (*Csa2G215490*), *CsGG2.2* (*Csa3G144190*), *CsGG3.1* (*Csa2G000110*), and *CsGG3.2*

¹http://www.icugi.org

(*Csa1G597050*). *CsGG3.1* alternative splicing produces two protein variants (CsGG3.1-1 and CsGG3.1-2). The six cucumber $G\gamma$ homologs were divided into two classes based on amino acid sequence alignments (**Figure 1**) and phylogenic methods (**Figure 2**). CsGG1, CsGG2.1, and CsGG2.2 belonged to the previously described type A, while CsGG3.1-1, CsGG3.1-2, and CsGG3.2 belonged to type C (Trusov et al., 2012).

CsGG3.2 Exhibited a Cold Inductive Expression Pattern

To investigate the cold responsive characteristic of CsGG genes, the expression patterns of these genes in leaves were detected by quantitative real-time polymerase chain reaction (qRT-PCR). As shown in **Figure 3**, CsGG1 transcript decreased from 0 to 24 h, followed by an increase at 168 h. CsGG2.1 and CsGG2.2 showed similar expression profiles, with transcript levels decreased at 4 h, while increased apparently at 168 h. CsGG3.1-1 showed relatively low transcript levels at most of the time, while the transcript of CsGG3.1-2 showed no response to cold treatment. Among the five selected time points, the transcript levels of CsGG3.2 climbed to the peak at 24 h and descended to a lower level at 168 h. These results demonstrate that CsGG3.2 might be involved in cold tolerance in cucumber.

Over-Expression of *CsGG3.2* Enhanced the Cold Tolerance of Transgenic Cucumbers

To investigate whether the CsGG3.2 function in chilling tolerance, transgenic cucumber plants over-expressing CsGG3.2 were constructed. Two lines (CsGG3.2-ox#1 and CsGG3.2-ox#2) with CsGG3.2 expression level about four fold higher compared with wild type (WT) cucumber (Figures 4A,B) were exposed to chilling stress to examine the chilling tolerance before and after cold acclimation. For non-acclimated plants, WT plants exhibited severe injury in their cotyledons, and even the first true leaves. Two transgenic lines CsGG3.2-ox#1 and CsGG3.2ox#2 showed lighter injuries (Figure 4C), and their CI indices significantly lower than WT, and were decreased by 18 and 21%, respectively (Figure 4E). For cold-acclimated plants, WT plants exhibited obvious injury in their cotyledons and first true leaves. Transgenic plants showed slight or no injury, and their CI indices were decreased by 30 and 34%, respectively (Figures 4D,F) compared with WT. These results suggest that the expression of CsGG3.2 enhanced the chilling tolerance of transgenic cucumber plants.

Over-Expression of *CsGG3.2* Enhanced *CBF* and *COR* Genes Transcripts Upon Exposure to Cold

Induction of *CBF* genes is important for chilling stress tolerance (Zhu et al., 2007). Therefore, we investigated the expression levels of *CBF* genes and their downstream target genes, *COR15b* and *KIN1* in *CsGG3.2* over-expressing transgenic and WT cucumber plants during cold acclimation. Cucumber *CBF* and *COR* genes were obtained by database searching using protein sequences of Arabidopsis CBFs, COR15b, and KIN1



as queries. *Csa5G174570* and *Csa3G751440* corresponded to the published *CsCBF1* (GenBank, DQ776899) and *CsCBF3* (GenBank, JQ900769), respectively. *Csa3G180260* coding a protein sharing high identities to AtCBF2, was named *CsCBF2*.



genome database under the following accession numbers: *CsCBF1*, Csa5G174570 (**A**); *CsCBF2*, Csa3G180260 (**B**); *CsCBF3*, Csa3G751440 (**C**). Data shown are averages \pm SE (n = 3). Three biological replicates were included for each experiment and 10 seedlings were included for each line. The asterisks indicate a significant difference between WT and *CsGG3.2* over-expressing plants (**p < 0.01).

Csa1G074950 and *Csa5G165870* coding late embryogenesis abundant (LEA) proteins were designated as *CsCOR15b* and *CsKIN1*, respectively. The transcript levels for *CsCBF1* and *CsCBF2* increased within 4 h of plants being exposed to cold stress. The levels of *CsCBF1* and *CsCBF2* expression was significantly higher in *CsGG3.2*ox plants compared with WT plants (**Figures 5A,B**). Levels of *CsCBF3* transcript were



FIGURE 6 | Relative gene expression of cucumber homologs *COR15b* (A), and *KIN1* (B) in *CsGG3.2* over-expressing T1-transgenic and WT plants under cold stress. Sequence data of *CsCOR15b* and *CsKIN1* can be found in the cucumber genome database under the accession number Csa1G074950 and Csa5G165870 respectively. Data shown are averages \pm SE (n = 3). Three biological replicates were included for each experiment and 10 seedlings were included for each line. The asterisks indicate a significant difference between WT and *CsGG3.2* over-expressing plants (*p < 0.05 and **p < 0.01).

relatively lower compared to that of *CsCBF1* and *CsCBF2*. *CsGG3.2* over-expressing significantly reduced the transcript level of *CsCBF3*, but had no effect at other time points (**Figure 5C**). Cold increases the *CsCOR15b* and *CsKIN1*, two known *CBF*-inducible COR genes, expression in both WT and *CsGG3.2*ox plants. And their expression levels were significantly up-regulated by *CsGG3.2* over-expressing (**Figure 6**).

Analysis of Antioxidant Enzymes Activity and Response of ROS and Malondialdehyde (MDA) in Transgenic Plants

The changes induced by cold in the activities of antioxidant enzymes and production of OFR, H_2O_2 , and malondialdehyde (MDA) in transgenic lines were compared with WT plants. Over-expression of *CsGG3.2* resulted in increased enzymatic activities of SOD, POD, CAT, and GR in transgenic plants under both normal and cold conditions (**Figures 7A–D**). And this



(D) Glutathione reductase (GR), one unit of enzyme activity defined as 1 nmol NADPH oxidized min⁻¹ per gram tissue. Data shown are averages \pm SE (n = 3). Three biological replicates were included for each experiment and 10 seedlings were included for each line. The asterisks indicate a significant difference between WT and *CsGG3.2* over-expressing plants (*p < 0.05 and **p < 0.01).

resulted in decreased accumulation of OFR and H_2O_2 in the transgenic plant in stressful environment compared with WT plants (**Figures 8A,B**). The increased detoxification of ROS led to reduced membrane lipid peroxidation, i.e., MDA production. MDA level was low (about 0.031 µmol g⁻¹ FW) under normal conditions in transgenic and WT plants. MDA content in WT plants increased to 0.060 µmol g⁻¹ FW under chilling stress, whereas with a lower increase, to only 0.048 µmol g⁻¹ FW and 0.050 µmol g⁻¹ FW respectively, was seen in the transgenic plants (**Figure 8C**).

DISCUSSION

Plant $G\gamma$ subunits are involved in a wide range of developmental and physiological processes, and have a high potential for crop improvements (Botella, 2012). In rice, *DEP1* and *GS3* are major quantitative trait loci for controlling seed size and panicle branching (Botella, 2012); RGG1 and RGG2 are upregulated upon salinity, harsh temperature, and ABA treatments (Yadav et al., 2012). Arabidopsis AGG1 and AGG2 are reported to be involved in osmotic stress and root development, and AGG3 is also found to be involved in regulation of organ size and stress response (Chakravorty et al., 2011; Li et al., 2012a,b; Thung et al., 2013). Similarly, the soybean GyIII subunit plays a role in ABA-dependent lateral root development (Choudhury and Pandey, 2013). Tomato SlGGB1 also mediates auxin and ABA signaling (Subramaniam et al., 2016). Here, we demonstrated that over-expression of CsGG3.2 promotes tolerance of cucumber seedlings to chilling stress. Moreover, multiple mechanisms appear to contribute to this increase in freezing tolerance, including alterations in gene expression associated with cold acclimation and the activation of antioxidative enzymes.

Chilling tolerance of plants was enhanced by cold acclimation. DREB1/CBFs are transcription factors regulating the expression of more than 100 COLD RESPONSIVE (COR) genes, and thus important for cold acclimation and chilling tolerance (Chinnusamy et al., 2007; Park et al., 2015). Constitutive expression of any one of the CBF genes in transgenic plants gives rise to strong constitutive expression of the COR genes and hence increased freezing tolerance in plants (Jagloottosen et al., 1998; Kasuga et al., 1999; Gilmour et al., 2004; Nakashima, 2006). Expression of CsCBF1 and CsCBF2 were upregulated in CsGG3.2ox plants (Figures 5A,B), supporting the proposed role of CsGG3 in promoting chilling tolerance. However, transcription levels of CsCBF3 were lower than that of CsCBF1 and CsCBF2 in both WT and transgenic plants (Figure 5C). This results may be due to the negative interaction between CBF genes, which is important for transient and tightly controlling their expression (Knight and Knight, 2012).

The expression of CBF genes activate many downstream genes that enhance plants chilling and freezing tolerance (Park et al., 2015). Many CBF-inducible genes have been cloned and characterized from Arabidopsis and other species. COR15a encodes a chloroplast-targeted polypeptide, which functions as cryoprotective protein preventing the formation of hexagonal II-phase lipids in the chloroplast stroma (Nakayama et al., 2007). The homolog of AtCOR15a, AtCOR15b has transcript profiles similar to that of AtCOR15a under cold stress (Wilhelm and Thomashow, 1993). And the transcript level of CbCOR15b from shepherd's purse (Capsella bursapastoris) was significantly upregulated under cold treatment, and over-expression of CbCOR15b enhanced cold tolerance in transgenic tobacco plants (Wu et al., 2012). KIN1 is another CBF downstream target gene from Arabidopsis encoding a 6.5 KDa polypeptide similarity to the type I fish antifreeze proteins. In Cucurbita crops, watermelon and pumpkin, transcripts of CmCOR15b, ClKIN1, and CmKIN1 significantly increased during cold stress condition, suggesting that they could contribute to the cold tolerance (Kang et al., 2009). Here, we showed that the transcript levels of cucumber CsCOR15b and CsKIN1 increased dramatically in response to low temperature (Figure 6), which are consistent with that previously found in watermelon and pumpkin (Kang et al.,



2009). Meanwhile, expression of *CsCOR15b* and *CsKIN1* was upregulated by *CsGG3.2* over-expressing (**Figure 6**), suggesting CsGG3.2 facilitates induction of *COR* genes expression, and cold tolerance.

Reactive oxygen species were produced and accumulated under cold stress, which damaged cell and yielded MDA (Achard et al., 2008; Swain et al., 2016). Antioxidant enzymes are important components of the ROS scavenging system in the plant cell, thus play a significant role in plant cold tolerance (Ahmad et al., 2010; Liu et al., 2010). It has been reported that activities of antioxidant enzymes in plants are increased under low temperature stress, which might be due to the upregulation of corresponding genes (Baek and Skinner, 2003; Soltész et al., 2011). In present study, the activity of SOD, POD, CAT, and GR enzymes in WT plants increased under cold condition, but the rates of increase were higher in transgenic plants (Figure 7). The increased detoxification of ROS led to reduced membrane lipid peroxidation, and could increase the chilling tolerance of transgenic plants. These results are in agreement with the previous studies where a decreased level of ROS production under cold stress has been reported in ICE1-ox cucumber (Liu et al., 2010).

Heterotrimeric G proteins play important roles in stress responses by interacting with Ca^{2+} channels in animals (Wang and Chong, 2010). Recently, plant G protein signaling found to be involved in cold signal transduction: activation of G protein by COLD1, triggered Ca^{2+} influx upon cold treatment, leading to a cytosolic Ca^{2+} signal (Ma et al., 2015). Ca^{2+} signal is postulated to activate a MAP kinase cascade, which then phosphorylate cold responsive TFs such as CAMTAs and ICE1/2 (Zhu, 2016). The exact mechanism of G protein-mediated plant cold response is not known yet. Here, our findings showed that CsGG3.2 positively regulates the expression of *CBF* genes and their regulon, as well as the activities of antioxidant enzymes, which lead to cold stress tolerance. The results suggest a critical role of CsGG3.2 in cucumber cold response, which will help in understanding the G protein-mediated cold signal transduction in plants.

AUTHOR CONTRIBUTIONS

XY, YaL, LB, and CH: conceived and designed the experiments. LB and YuL: performed the experiments. LB: analyzed the data. LB, YM, and YY: contributed reagents/materials/analysis tools. LB, YuL, and AA: wrote the paper.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpls.2018.00488/ full#supplementary-material

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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