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Full Length Article

Cloning, Characterization and Expression Analysis of *GbWRKY11*, a Novel Transcription Factor Gene in *Ginkgo biloba*

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Abstract

WRKY transcription factors are involved in diverse life processes in plants. Thus far, these proteins have not been reported in *Ginkgo biloba*. In this study, we cloned *GbWRKY11*, a novel WRKY transcription factor gene, from *G. biloba*. The cDNA of *GbWRKY11* is 1,707 bp in length and encodes a putative protein of 402 amino acids. The GbWRKY11 protein has an estimated molecular weight of 43.64 kDa and belongs to the WRKY IId group. The genomic DNA of *GbWRKY11* contains two introns and three exons. *GbWRKY11* gene is ubiquitously expressed in all tested tissues but preferentially expressed in female flowers. The *GbWRKY11* transcript is upregulated in response to salicylic acid, ethephon, and abscisic acid but repressed by methyl jasmonate, salinity, cold and heat. Thus, *GbWRKY11*, a newly identified *G. biloba* WRKY transcription factor, is apparently involved in multiple signaling pathways in response to abiotic stresses. This study provides a basis for further research on the function of *GbWRKY11*.

Keywords: *GbWRKY11*; *Ginkgo biloba*; Transcription factor; Abiotic stress

Introduction

Ginkgo biloba, usually referred to as a "living fossil," is the sole surviving species of the previously large plant division Ginkgophyta (Van Beek and Montoro, 2009). *G. biloba*, which experienced million years of complicated climate conditions, not only shows strong adaptability but also minor morphological changes; this characteristic could be due to the ability of *G. biloba* to adapt to the environment (Deng *et al*., 2006). *G. biloba* responds to environmental signals by modulating gene expression, regulating protein content or activity, altering metabolite levels, and changing the homeostasis of ions (Eyidogan *et al*., 2012). Thus, *G. biloba* could be a suitable model plant for studies on regulating a range of abiotic and biotic stresses.

A crucial aspect of plant responses to all types of stresses is the transcriptional control of defense-related gene expression (Singh *et al*., 2002). Transcription factors, including MYB, ethylene response factor, WRKY proteins, basic-domain leucine zipper, and other zinc finger factors, regulate the expression of diverse stress-responsive genes (Carlberg and Molnár, 2014).

The WRKY transcription factors are characterized by their DNA-binding domain of about 60 amino acids. The WRKY domain is composed of a conserved WRKYGQK peptide at the N-terminal and a typical zinc finger motif at the C-terminal, with the zinc finger structure as either C_2H_2 ($CX_{4\text{-}5}CX_{22\text{-}23}HX_1H$) or C_2HC ($CX_7CX_{23}HX_1C$). The WRKYGQK motif and the C_2H_2 or C_2HC zinc finger are essential for DNA–protein interaction (Llorca *et al*., 2014). The WRKY protein contains one or two copies of the WRKY domain. These transcription factors are classified into three subgroups based on the number of WRKY domain and the structure of zinc finger. Group I transcription factors contain two WRKY domains and a C_2H_2 zinc finger motif. Groups II and III contain only one WRKY domain, with zinc finger motifs of C_2H_2 and C_2HC, respectively (Eulgem *et al*., 2000). Group II WRKY transcription factors are further categorized into five small subgroups (IIa, IIb, IIc, IId, and IIe) based on their amino acid sequences (Rushton *et al*., 2010).

WRKY transcription factors mainly exist in plants and regulate multiple biological processes. These transcription factors are assumed to regulate multiple stress-responsive genes by interacting with the W box (TTGACC/T) in the promoters of their target genes. For example, single gene interference or co-silencing of *NaWRKY3* and *NaWRKY6* promotes the susceptibility of tobacco to herbivore damage by reducing the accumulation of volatile sesquiterpene and jasmonate (Skibbe *et al*., 2008). In *Arabidopsis thaliana*,

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overexpression of *AtWRKY3* and *AtWRKY4* improves the resistance of the plant to fungal diseases caused by *Botrytis* (Lai *et al*., 2008). Moreover, the expression of *WRKY* genes can be induced by drought, wounding, and plant hormones and triggers signal transduction cascades (Banerjee and Roychoudhury, 2015). The tolerance of rice to high temperatures and drought can be enhanced by overexpressing *OsWRKY11* (Wu *et al*., 2009). In *Musa spp*., the expression level of *MusaWRKY71* is upregulated after the plant suffers from cold, drought, salt, ABA, H_2O_2, ethephon (ETH), salicylic acid (SA), and methyl jasmonate (MeJA) stresses (Shekhawat *et al*., 2011).

Several gene studies have implicated WRKY proteins in plant secondary metabolism. Kato *et al*. (2007) isolated and identified the transcription factor *CjWRKY1* from *Coptis japonica* and found a positive correlation between the expression of this gene and all the examined berberine biosynthesis genes, indicating that *CjWRKY1* is involved in berberine biosynthesis. Ma *et al*. (2009) also isolated *AaWRKY1* from the glandular secretory trichomes of *Artemisia annua*, in which artemisinin is synthesized and sequestered. *AaWRKY1* activates the expression of the majority of artemisinin biosynthesis genes, thereby suggesting that *AaWRKY1* is a necessary positive regulator in artemisinin biosynthesis. Recently, Li *et al*. (2013) have reported that *TcWRKY1* regulates taxol biosynthesis in *Taxus chinensis*.

Thus far, no WRKY genes have been isolated and characterized from *G. biloba* because of limited sequence information. We previously performed transcriptome analysis of *G. biloba* by using Illumina HiSeq™2500 sequencing platform. The dataset provides abundant information on the sequences of transcription factor genes, which can be used for full-length cDNA cloning and functional studies. In the present study, we cloned and characterized *GbWRKY11*, a novel WRKY transcription factor gene, from *G. biloba*. *GbWRKY11* is preferentially expressed in female flowers and induced by phytohormones, such as SA, ETH, and ABA, but repressed by MeJA, salinity, cold, and heat. This work is the first to report a WRKY transcription factor in *G. biloba*.

Materials and Methods

Plant Material and Stress Treatments

G. biloba grafts of 14 years old were grown in Botanical Garden of Yangtze University, China. In order to study the tissue expression patterns of *GbWRKY11*, the leaves, stems, roots, male and female flowers of *G. biloba* graft were collected for RNA extraction.

The cultured callus, initiated from mature zygotic embryos of *G. biloba*, were cultured on liquid MS basal medium supplementing with 2 mg/L 6-benzyladenine (6-BA) and 1.5 mg/L naphthaleneacetic acid (NAA) on a rotary shaker at 100 rpm, in the light and at 25 ± 1°C. The suspension cultures were subcultured every 2 weeks and after four subcultures the differential was omitted. In the experiments for testing induction by various elicitors, the callus were dipped into the treatment such as 100 μmol/L methyl jasmonate (MeJA), 100 μmol/L abscisic acid (ABA), 100 μmol/L salicylic acid (SA), 40 μmol/L ethephon (ETH) and 200 mmol/L sodium chloride (NaCl), respectively, using the callus without any treatment as control. The cold and heat treatments were applied by placing the callus lines in a 4°C and 40°C rotary shaker while the control in a 25°C growth room. The callus samples were harvested 0, 3, 6, 12, 24, 48 and 72 h after treatment and immediately frozen in liquid nitrogen, and kept at −80°C prior to total RNA extraction.

RNA and DNA Extraction

Total RNA was extracted from different organs and callus of all the treatments using the CTAB method (Cai *et al*., 2007; Liao *et al*., 2004). Genomic DNA was extracted from the leaves of ginkgo grafts following the CTAB method (Xu *et al*., 2008).The quantity and quality of DNA and RNA were detected by spectrophotometer analysis and agarose gel electrophoresis.

Cloning of cDNA and Genomic DNA of *GbWRKY11*

First-strand cDNA was synthesized using PrimeScript™ 1st Strand cDNA Synthesis Kit (TaKaRa, Dalian, China) according to the instruction book. Using a pair of *GbWRKY11*-specific primers WRKY11-FP (5′-GGAGGAGAATGACGAAGAGTGC-3′) and WRKY11-RP (5′-GTCTTTCTGCTCCTACATTTGATT-3′), the cDNA and genomic DNA of *GbWRKY11* containing full open reading frame were amplified using the first-strand cDNA and genomic DNA as the temple, respectively. The PCR product was purified and cloned into pMD19-T vector (TaKaRa, Dalian, China), followed by sequencing for confirmation.

GbWRKY11 Transcript Analysis by Real-time PCR (qRT-PCR)

The RNA samples were reversely transcribed using the PrimeScript™ RT-PCR kit (TaKaRa, Dalian, China). The housekeeping gene, namely glyceraldehyde-3-phospate dehydrogenase gene (*GbGAPDH*, L26924), was used as a reference gene with primers of GAPDH-U (5′-GGTGCCAAAAAGGTGGTCAT-3′) and GAPDH-D (5′-CAACAACGAACATGGGAGCAT-3′). Gene-specific primers of WRKY11-U (5′-TGTAAGAGGTTGCCCTGCTAGA-3′) and WRKY11-D (5′-TGGTTATGTTCTCCCTCGTATGT-3′) for *GbWRKY11*. The experiments were repeated independently with three biological replicates using SYBR® Premix Ex Taq™ II (TaKaRa, Dalian, China) in the Applied

Biosystems 7500 Realtime PCR systems (Foster City, CA, USA). Relative expression level was calculated using the method described by Livak and Schmittgen (2001).

Bioinformatic Analysis

The sequence was analyzed by the bioinformatic software on websites (http://www.xpasy.org and http://www.ncbi.nlm.nih.gov). Plant WRKY protein sequences were retrieved from NCBI GenBank. Sequence alignment was performed using ClustalX 2.0 and phylogenetic tree was constructed by neighbor-joining method using MEGA version 5. A bootstrap statistical analysis was carried out with 1000 replicates.

Results

Cloning and Sequence Analysis of *GbWRKY11*

The WRKY domain (PF03106) was used to search for the transcriptome data of *G. biloba*. We obtained a sequence with high similarity to the known WRKY transcription factors of other plants. Using a pair of specific primers based on this sequence, we performed PCR using cDNA as the template and a 1,707 bp *GbWRKY11* clone was obtained. Sequence analysis revealed that *GbWRKY11* cDNA contained 1,209 bp-length open reading frame, as well as 201 and 297 bp long 5′ and 3′ untranslated regions, respectively. We compared the identities of this transcription factor with the nucleotide sequences of other plants in the NCBI database and found 92, 88, 82 and 83% similarities to *PmWRKY104* in *Pinus monticola*, *GhWRKY61* in *Gossypium hirsutum*, *BrWRKY15* in *Brassica rapa*, and *AtWRKY7* in *A. thaliana*, respectively.

The specific primers WRKY11-FP and WRKY11-RP were synthesized using genomic DNA as template and used for PCR to obtain the genomic DNA of *GbWRKY11*. The amplification product is 2,794 bp long (Fig. 1). Comparison of the cDNA and DNA sequences revealed that the genomic DNA of *GbWRKY11* contains two introns with lengths of 258 and 829 bp. The two introns contain the conserved GT/AG splicing site and high AT (67.0% of intron 1 and 68.2% of intron 2), which are typical characteristics of plant introns. In addition, both introns are of R-type and their insertion position was found in the codon of arginine between the nucleotide AG and A (Wu *et al*., 2005). The second intron interrupted the encoded region of the WRKY domain, and this feature is common in *Arabidopsis* WRKY (Eulgem *et al*., 2000). These results demonstrated that the typical intron splicing site may play a crucial role in evolution.

Characterization and Phylogenetic Analysis of the GbWRKY11 Protein

The GbWRKY11 protein encodes a 402 amino acid residue, harbors one WRKY domain containing 60 amino acids, and presents one C_2H_2-type zinc-finger motif. Hence,

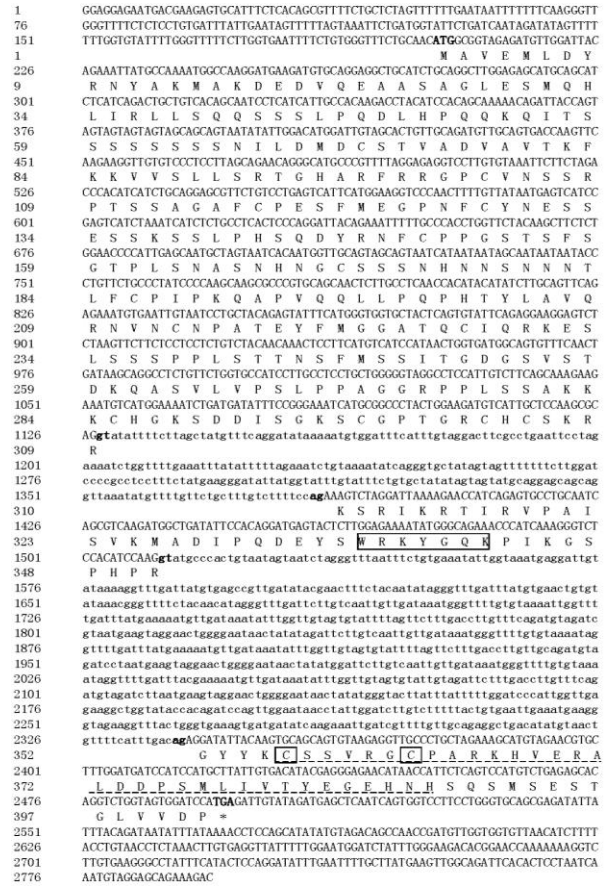

Fig. 1: The full-length cDNA, intron and deduced amino acid sequence of *GbWRKY11* gene. The exons sequence is indicated in capital letter and the intron is indicated in lowercase. The start condon (ATG), the stop (TAG) and putative exon-intron splicing sites (gt/ag) are shown by bold letters. The WRKYGQK motif and the C and H residues in the zinc-finger motif ($CX_4CX_{23}HX_1H$) are boxed. The zinc-finger motif ($CX_4CX_{23}HX_1H$) is marked by dashed underline

GbWRKY11 belongs to the group II of the WRKY transcription factor family (Rushton *et al*., 2010). The molecular weight of the GbWRKY11 protein is 43.64 kDa, and the theoretical isoelectric point is 9.19. The GbWRKY11 protein shared 66%, 67%, 68%, 76% and 77% identities to GsWRKY11 of *Glycine soja*, GhWRKY11-1 of *G. hirsutum*, VpWRKY11 of *Vitis pseudoreticulata*, EgWRKY11 of *Elaeis guineensis*, and PdWRKY11 of *Phoenix dactylifera*, respectively. As this *G. biloba* WRKY is closely correlated with WRKY11, we named the protein as GbWRKY11 (GenBank accession number: KP987206).

Sequence analysis showed that the predicted GbWRKY11 protein contains two putative nuclear localization signal (NLS) sequences (^{83}KKVVSLLSRTGHARFRR^{100} and ^{306}KRRK^{310}, Fig. 2). Hence, *GbWRKY11* is likely to localize to the nucleus.

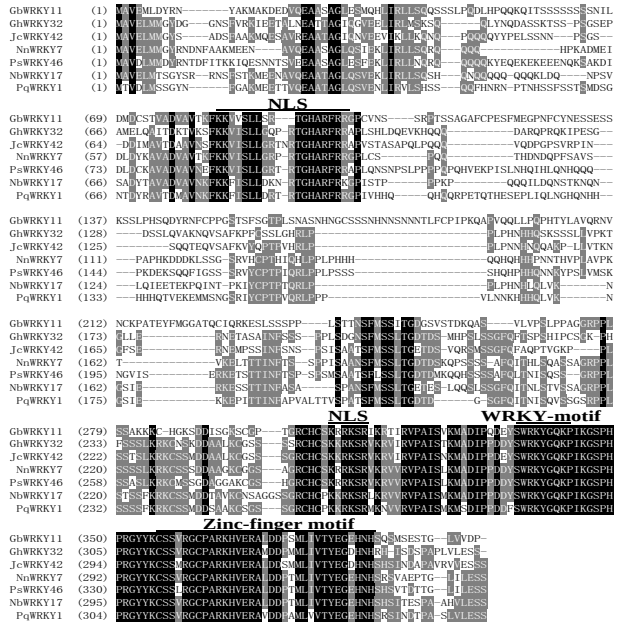

Fig. 2: Sequence multi-alignment of the deduced GbWRKY11 protein with other WRKYs. The completely identical amino acids are indicated with white foreground and black background. The conserved amino acids are indicated with white foreground and grey background. Non-similar amino acids are indicated with black foreground and white background. The WRKY-motif, zinc-finger motif ($CX_5CX_{23}HX_1H$) and two putative nuclear localization signals are uplined. The accession numbers of WRKY proteins and translation of their names are shown as follows, GbWRKY11: *Ginkgo biloba* ; GhWRKY32: *Gossypium hirsutum* AGV75940.1; JcWRKY42: *Jatropha curcas* AGQ04232.1; NnWRKY7: *Nelumbo nucifera* XP_010270802.1; PsWRKY46: *Papaver somniferum* AFU81789.1; NbWRKY17: *Nicotiana benthamiana* AIR74899.1; SlWRKY7: *Solanum lycopersicum* XP_004238130.1; StWRKY2: *Solanum tuberosum* ABU49721.1; SiWRKY7: *Sesamum indicum* XP_011074403.1; PqWRKY1: *Panax quinquefolius* AEQ29014.1; VvWRKY7: *Vitis vinifera* XP_002284966.1

GbWRKY11 showed high protein sequence identities (41%–48%) to Group IId WRKY proteins, including GhWRKY32 of *G. hirsutum*, JcWRKY42 of *Jatropha curcas*, PqWRKY1 of *Panax quinquefolius*, NbWRKY17 of *Nicotiana benthamiana*, PsWRKY46 of *Papaver somniferum*, and NnWRKY7 of *Nelumbo nucifera*. As shown in Fig. 2, the WRKY domain and nuclear localization sites were strictly conserved but litter sequence conservation existed outside these regions.

A phylogenetic tree was constructed with 18 WRKY proteins, including GbWRKY11 and other WRKY transcription factors. The results showed that GbWRKY11 was closely related to PsWRKY of *Picea sitchensis*, AtWRKY11 of *A. thaliana*, NnWRKY7 of *N. nucifera*, and

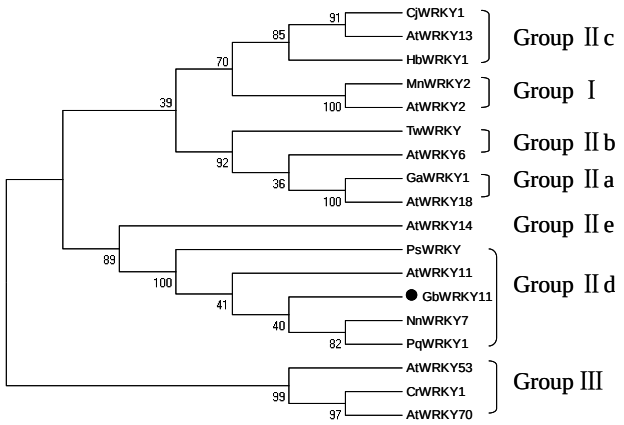

Fig. 3: Phylogenetic tree of the sequences of *GbWRKY11* and other plants WRKY protein. The numbers at each node represented the bootstrap values (with 1000 replicates). The accession numbers of WRKY proteins and translation of their names are shown as follows,GbWRKY11:*Ginkgo biloba*; CjWRKY1:*Coptis japonica var. dissecta* BAF41990.1; AtWRKY13: *Arabidopsis thaliana* AEE87071.1; HbWRKY1: *Hevea brasiliensis* ADF45433.1; MnWRKY2: *Morus notabilis* XP_010092241.1; AtWRKY2: *Arabidopsis thaliana* AED96743.1; TwWRKY: *Taxus wallichiana var. chinensis* AEW91476.1; AtWRKY6: *Arabidopsis thaliana* AEE33948.1; GaWRKY1: *Gossypium arboreum* AAR98818.1; AtWRKY18: *Arabidopsis thaliana* AEE85961.1; AtWRKY14: *Arabidopsis thaliana* AEE31256.1; PsWRKY: *Picea sitchensis* ADE77495.1; AtWRKY11: *Arabidopsis thaliana* AEE85928.1; NnWRKY7 Nelumbo nucifera XP_010270802.1; PqWRKY1:*Panax quinquefolius* AEQ29014.1; AtWRKY53: *Arabidopsis thaliana* AEE84809.1; AtWRKY70: *Arabidopsis thaliana* AEE79517.; CrWRKY1:*Catharanthus roseus* HQ646368

PqWTKY1 of *P. quinquefolius*. Thus, GbWRKY11 was clustered in the group IId of the WRKY family (Fig. 3).

Expression Profile of *GbWRKY11* in Different Tissues

Some studies revealed that WRKY transcripts were present and exhibited varied expression levels in all tissues (Zheng *et al*., 2011; Wang *et al*., 2013; Song and Nan, 2014; Yang *et al*., 2015). As such, *GbWRKY11* expression patterns in tissues were systematically analyzed through qRT-PCR method. As shown in Fig. 4, *GbWRKY11* transcripts were detected in all tissues but their expression levels varied in tissues of different organs. The highest gene expression level was observed in the female flowers, whereas the lowest level was detected in the roots and male flowers. The expression level of *GbWRKY11* was significantly higher in the stems than that in the leaves but still lower than that in the female flowers.

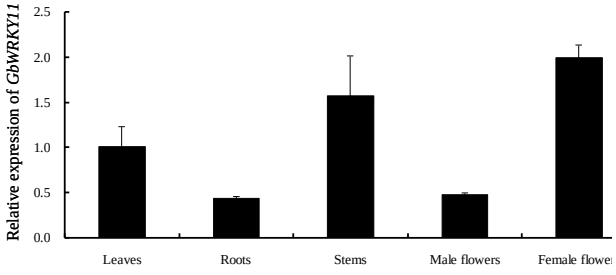

Fig. 4: Expression profiles of GbWRKY11 in G. biloba different tissues. Total RNA samples were isolated from leaves, roots, stems, male flowers and female flowers respectively. The expression levels of each tissue were normalized to *GAPDH* gene. The gene level of leaves was set to 1, and those tissues were accordingly accounted and presented as the relative fold changes. At least three biological replicates were carried out for each sample and the error bars indicated the standard errors of the mean

Expression Patterns of *GbWRKY11* under Abiotic Stress

The callus lines of *Ginkgo* were subjected to cold (4°C), heat (40°C), and salt (NaCl) treatments to investigate the expression of *GbWRKY11* under various abiotic stresses. The results are shown in Fig. 5. The transcript level of *GbWRKY11* minimally changed in response to salt stress at 3 h post treatment (hpt) and then sharply decreased thereafter (Fig. 5A). Cold stress gradually reduced the mRNA level of *GbWRKY11* until the end of the experiment (Fig. 5B). Similarly, heat stress progressively repressed *GbWRKY11* expression (Fig. 5C). These results showed that abiotic stresses, except salt stress, repressed *GbWRKY11* expression. Thus, we deduced that *GbWRKY11* can repress salt-, cold- and heat-response genes.

Effects of Signaling Molecules on *GbWRKY11* Transcription

Signaling molecules, such as SA, MeJA, ABA, and ETH, can regulate the expression of defense-related genes in response to biotic and abiotic stresses (Bari and Jones, 2009). The expression levels of *GbWRKY11* were analyzed through qRT-PCR in *Ginkgo* callus treated with SA, MeJA, ABA, and ETH to determine the involvement of the gene in signaling pathways. The expression of *GbWRKY11* was rapidly upregulated in response to exogenous application of ETH at 3 hpt, reached the highest level at 12 hpt (8.8-fold relative to mock-treated control samples), and reduced thereafter until the end of the experiment (Fig. 6A). The transcript level of *GbWRKY11* minimally changed in response to SA treatment at 3 hpt, sharply decreased between 6 and 12 hpt, reached the highest level at 24 hpt (6.3-fold relative to the mock-treated control samples), and then gradually decreased until the end of the experiment (Fig. 6B). *GbWRKY11* transcript levels were sharply enhanced in response to ABA treatment at 6 hpt, reached the highest level

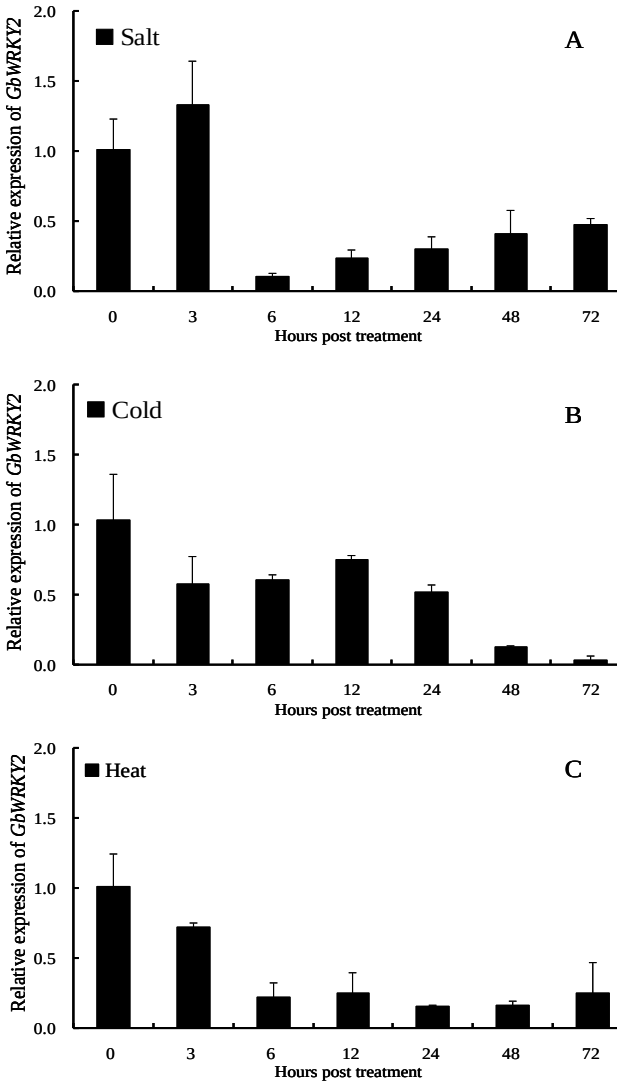

Fig. 5: Expression profiles of *GbWRKY11* in response to salt (A), cold (B) and heat (C) treatment. All samples were collected at the indicated time points. For each treatment, the expression levels of each time point were normalized to *GAPDH* gene. The gene expression level at the onset of stress treatments was set to 1, and those at other points were accordingly accounted and presented as the relative fold changes. At least three biological replicates were carried out for each time point and the error bars indicated the standard errors of the mean

at 6 hpt (3.1-fold relative to mock-treated control samples), and then decreased until the completion of the treatment (Fig. 6C). Meanwhile, the transcript levels of *GbWRKY11* gradually decreased in response to MeJA until the end of the experiment (Fig. 6D). These results show that *GbWRKY11* may regulate the responses of *G. biloba* to SA, ETH, and ABA treatments. Basing from these results, we speculated that *GbWRKY11* may play a crucial role in the phytohormone-regulated defense-related responses of *G. biloba*.

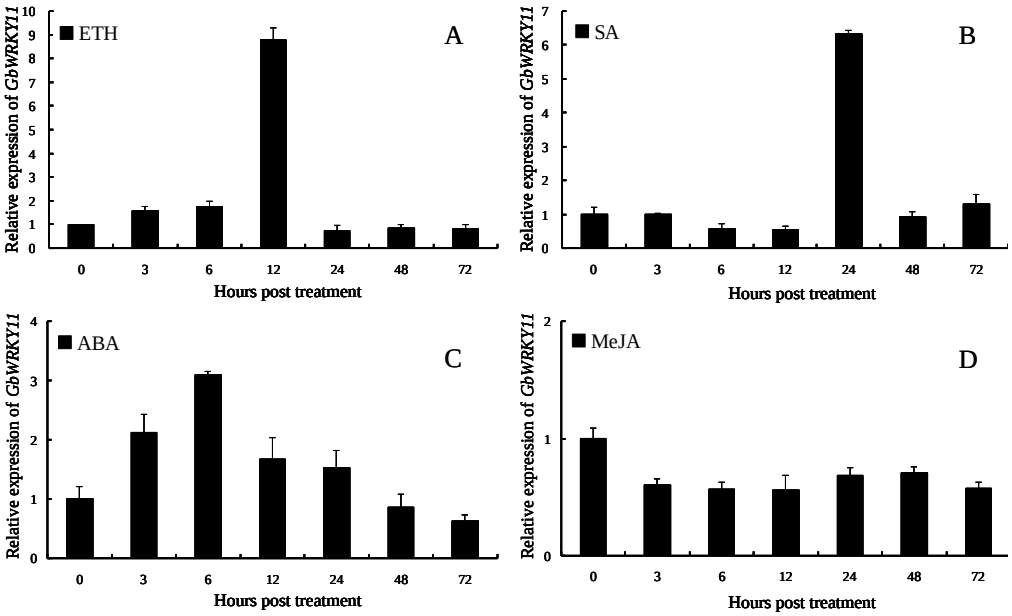

Fig. 6: Expression profiles of *GbWRKY11* in response to ETH (A), SA (B), ABA (C) and MeJA (D) treatment. The gene expression level at the onset of stress treatments was set to 1, and those at other points were accordingly accounted and presented as the relative fold changes. At least three biological replicates were carried out for each time point and the error bars indicated the standard errors of the mean

Discussion

Numerous studies indicate that plant WRKYs play an intricate role in plant adaptation to various stresses (Banerjee and Roychoudhury, 2015). A large number of WRKY genes have been isolated and characterized in various plant species, such as in *Arabidopsis* (Eulgem *et al*., 2000), *Zea mays* (Wei *et al*., 2012), *Gossypium* (Dou *et al*., 2014), *Medicago truncatula* (and Nan, 2014), *Cucumis sativus* (Ling *et al*., 2011), *B. rapa* (Tang *et al*., 2014), *Vitis vinifera* (Wang *et al*., 2014), *Carica papaya* (Pan and Jiang, 2014), *Coffea arabica* (Ramiro *et al*., 2010), *Populus* (Jiang *et al*., 2014), *Hevea brasiliensis* (Li *et al*., 2014), *Coptis japonica* (Kato *et al*., 2007), and *Catharanthus roseus* (Yang *et al*., 2013). However, knowledge on WRKYs of *G. biloba* remains limited. WRKY is a large family of transcription factors, most of which have not yet been elucidated, particularly in non-model plants.

In this study, we isolated *GbWRKY11*, a novel WRKY gene, from *G. biloba.* Phylogenetic analysis results demonstrated that *GbWRKY11* belongs to the group IId of the WRKY transcription factor family. An increasing number of transcription factors belonging to group IId WRKY have been isolated and identified from several plants. For example, *PqWRKY1*, which responds to MeJA, regulated osmotic stress responses and triterpene ginsenoside biosynthesis in *P. quinquefolius* (Sun *et al*., 2013). In *Brassica rapa*, *BrWRKY7*, which is the homolog of *AtWRKY7*, was induced 6 h after SA treatment (Kim *et al*., 2008). Further research revealed that all *Arabidopsis* WRKY group IId proteins, including *AtWRKY11*, contain the calmodulin-binding and RTGHARFRR[A/G]P domains (Park *et al*., 2005). *AtWRKY11* is a negative regulator that resists *Pseudomonas syringae* pv. tomato and is involved in jasmonic acid (JA)-dependent responses (Journot–Catalino *et al*., 2006). Although these two domains were not found in *GbWRKY11*, the possibility that *AtWRKY11* and *GbWRKY11* exhibit similar functions cannot be excluded. Two predicted NLS sequences were also uncovered using the PSORT program, which implied that *GbWRKY11* may function in the nucleus.

GbWRKY11 is highly expressed in female flowers and stems. The expression pattern of *GbWRKY11* is consistent with that of *HbWRKY1*, a negative transcription regulator of small rubber particle protein, which is involved in biosynthesis of natural rubber in *H. brasiliensis* (Wang *et al*., 2013). This result implied that *GbWRKY11* may play a crucial role in the growth of the female flower and stems in *G. biloba.*

The expression of WRKY proteins, which regulate diverse developmental and defense-related responses, can be activated by a series of signaling molecules, such as ETH, SA, MeJA, and ABA (Jiang and Yu, 2015). The current study revealed that *GbWRKY11* expression was induced by ETH, SA, and ABA but was repressed by MeJA. Increasing studies have demonstrated that WRKY genes play vital roles in both JA- and SA-mediated signaling pathways. For example, in *Capsicum*, *CaWRKY30* expression was rapidly upregulated by SA treatment but downregulated by MeJA (Zheng *et al*., 2011). In *Helianthus*,

the expression of *HaWRKY3* and *HaWRKY76* was significantly upregulated by SA but downregulated by MeJA (Giacomelli *et al*., 2010). Similarly, *AtWRKY70* expression in *Arabidopsis* was induced by SA but repressed by MeJA (Li *et al*., 2004). Consistent with these findings, *GbWRKY11* expression was also upregulated by SA and downregulated by MeJA, suggesting that *GbWRKY11* may play similar roles to that of *HaWRKY3*, *HaWRKY76*, *CaWRKY30*, or *AtWRKY70*. Previous studies have demonstrated that WRKY proteins may act as activators in ABA signaling (Antoni *et al*., 2011). For example, in *Larrea tridentate*, *LtWRKY21* is an activator that controls the expression of ABA-regulated genes (Zou *et al*., 2007). Sun *et al.* (2014) also reported that 13 numbers of WRKY family in *O. sativa* were upregulated by ABA. Pathways involving MeJA and ET are considered to be mainly effective against necrotrophic pathogens, insects, and wounding (Kunkel and Brooks, 2002). Thus, little attack of pathogens and insects in ginkgo may be due that WRKY protein, such as *GbWRKY11*, played important roles in defense responses by mediating MeJA and ET signaling. However, the molecular mechanism of *GbWRKY11* in response to signaling molecules requires further study.

Conclusion

As *GbWRKY11* expression was upregulated by SA, ETH, and ABA and repressed by MeJA, we infer that *Gb*WRKY11 regulates defense-related signaling pathways. We established the binary expression vector for overexpression of *Gb*WRKY11 for further studies. Further research on the genetic transformation of this gene in *G. biloba* callus should be performed. The present study provides a basis to elucidate whether the upregulated gene expression can enhance the ability of plants to resist abiotic stresses.

Acknowledgments

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