Molecular Cloning and Sequence Analysis of GbWRKY31, A Novel Transcription Factor Gene from Ginkgo biloba

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Abstract

WRKY proteins are a class of plant-specific transcription factors involved in stress response signaling pathways. In this work, a novel WRKY transcription factor gene, named after GbWRKY31, was isolated by RT-PCR method from the Ginkgo biloba. The full-length cDNA of GbWRKY31 was 1738 bp and contained a 1281bp open reading frame (ORF) encoding 427 amino acids. The estimated isoelectric point (pI) and molecular weight of the putative GbWRKY31 protein were 9.10 and 47.05 kDa, respectively. Homology analysis indicated that the deduced GbWRKY31 protein was highly homologous to other WRKY Proteins from different species and had a typical WRKY conserved domain database. Phylogenetic analysis showed that GbWRKY31 belongs to the WRKY IIb group. The results suggest that GbWRKY31 belongs to WRKY families and may be involved in the modulation of senescence and defense in G. biloba.

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Keywords

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Introduction

Ginkgo biloba, the oldest species in the world, has been growing for nearly 200 million years on the earth (He et al., 2009). It has many good characters, such as disease-resistance, drought-resistance (Cheng et al., 2013). Recent studies have shown that WRKY transcription factors were play an important role in plant growth involved in physiological metabolism process of biological signal stimulation and defense responses against pathogens.

It has been reported that WRKY transcription factors are crucial regulators in the transcription and disease resistance (Eulgem and Somssich, 2007). For example, AtWRKY70 in Arabidopsis thaliana, appears to affect the balance between signaling branches promoting SA-dependent and suppressing JA-dependent responses (Li et al., 2006); The rice carrying OsWRKY71 gene had a stronger resistance to Xanthomonas oryzae, in addition, the expression of disease resistance related gene was also significantly increased (Liu et al., 2007).

The WRKY protein includes one or more WRKY domains, which were composed of conserved WRKYGQK peptide and typical zinc finger motif, CX₄₋₅CX₂₂₋₂₃HXH (C₂H₂) or CX₇CX₂₃HXC (C₂HC), at the N-terminal and the C-terminal separately (Eulgem et al., 2000). According to the quantity of WRKY domain and the zinc finger motif, WRKY gene families can be divided into three groups: Group I included two WRKY domains with a C₂H₂ zinc-finger structure; Group II and III consist of only one WRKY domain with a C₂H₂ and a C₂HC zinc-finger structure, respectively. Meanwhile, the group II WRKY genes were further divided into IIa, IIb, IIc, IId, and Ile based on their primary amino acid sequence (Rushton et al., 2012).
In terms of molecular biology, WRKY proteins perform themselves function through specifically combining with the W-box (5'-TTGACC/T-3'), which is the cis-element and the minimal consensus required for specific DNA binding (Rushton et al., 1996; Ciolkowski et al., 2008). W-box sequences generally exist in promoter region involving in stress response. Moreover, WRKY genes promoter regions also often have W-box (Maleck et al., 2000). In recent years, a lot of WRKY transcription factors had been identified, and then discovered the association with secondary metabolism. The function of AtWRKY75 has been confirmed in regulating Pi starvation responses (Devaiah et al., 2007). TcWRKY1 participates in regulation of taxol biosynthesis in Taxus chinensis cells (Li et al., 2013). Previously, there are few WRKY genes that had been isolated and characterized from G. biloba. At present, transcriptome analysis of G. biloba was performed by our laboratory research group designed through using Illumina HiSeq™2500 sequencing platform. Based on the transcriptome data, we cloned and characterized GbWRKY31 from G. biloba.

Materials and methods

Plant material and reagents

The leaves of ginkgo were collected from Botanical Garden of Yangtze University, China, and stored at -80 °C refrigerator immediately. Both the primers synthesis and DNA sequencing were performed by Shanghai Sangon Biotechnology Company, in China. Agarose Gel DNA purification Kit Ver.4.0, pMD19-T vector kit, AMV Reverse Transcriptase, RNase, PrimeScript™ 1st Strand cDNA Synthesis kit PrimeScript™ RT-PCR kit and Taq DNA polymerase were purchased from Takara Company, Dalian, China.

RNA extraction and reverse transcription

Total RNA was isolated from plant frozen plant tissues using the MiniBEST Plant RNA Extraction kit (TaKaRa, Dalian, China). According to the instructions of PrimeScript™ 1st Strand cDNA Synthesis Kit (TaKaRa, Dalian, China), first-strand cDNA was synthesized.

Cloning of GbWRKY31

The specific primer GbWRKY31-F (5’-AAGGTTTCCC AATGCGGATAAG-3’) and GbWRKY31-R (5’-AGTG CCGCAGTGAAGTTGGGA-3’) were designed using the software of DNAMAN6. The PCR was performed using the one-step RT-PCR kit (TaKaRa, Dalian, China) under the following conditions: per-denaturation at 94°C for 3 min; denaturation at 94°C for 30s, anneal at 58°C for 30s, and extension at 72°C for 40s, by 35 cycles; extend at 72°C for 10 min. The PCR product was purified and cloned into pMD19-T vector kit (TaKaRa, Dalian, China), followed by sequencing for confirmation.

Bioinformatic analysis

The sequence was analyzed by the bioinformatics software on websites (http://www.xpasy.org and http://www.ncbi.nlm.nih.gov), 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 6.0. The bootstrap statistical analysis was carried out with 1000 replicates.

Results

Cloning and sequence analysis of GbWRKY31

The cDNA sequence of GbWRKY31 gene was obtained according to a pair of specific primers and total RNA reverse transcription product. The length of GbWRKY31 was 1738 bp, and the gene contained 1281 bp-length open reading frame and encoding 427 amino acids (Fig. 1). We compared the nucleotide sequence of GbWRKY31 with the nucleotide sequences of other plants acquired from the NCBI database and found the nucleotide sequence of GbWRKY31 had high similarity with WRKY genes of other plants (Table 1).

Characterization of the deduced GbWRKY31 protein

The GbWRKY31 protein encodes 552 amino acids (Fig. 1). Using the online website (http://web.expasy.org/compute_pi/), the molecular weight could be known and the theoretical isoelectric point of the GbWRKY31 protein were 47.05 kDa and 9.10, respectively. Using BLAST search of GeneBank and Vector NTI, the Fig. 2 shows that the similarity of the GbWRKY31 protein with other WRKY proteins was made. Sequencing analysis indicated that the predicted GbWRKY31 protein contains WRKY-motif and Zinc-finger motif. GbWRKY31 protein was 43-44% similarity to Group IIb WRKY proteins including EgWRKY31 of Eucalyptus grandis, PeWRKY31 of Populus euphratica, PkWRKY of Pierorhiza kurrooa, MaWRKY31 of Musa acuminata, CsWRKY31 of Cucumis sativus, VrWRKY31 of Vigna radiata, GmWRKY23 of Glycine max and GsWRKY6 of Glycine soja.
Table 1. Nucleotide sequence of GbWRKY31 similarity to the WRKY genes of other plant species.

<table>
<thead>
<tr>
<th>Species</th>
<th>GenBank no.</th>
<th>Identity</th>
<th>E-value</th>
</tr>
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<tbody>
<tr>
<td>Ziziphus jujuba</td>
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<td>97%</td>
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<tr>
<td>Gossypium hirsutum</td>
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<td>1e-05</td>
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<td>93%</td>
<td>1e-05</td>
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<td>Brassica rapa</td>
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<tr>
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<tr>
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<td>Solanum tuberosum</td>
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<td>74%</td>
<td>1e-15</td>
</tr>
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</table>

Note: The nucleotide sequence of GbWRKY31 was 97%, 93%, 93%, 88%, 81% similarity to ZjWRKY72 in Ziziphus jujuba, GhWRKY90 in Gossypium hirsutum, GhWRKY80 in Gossypium hirsutum, EgWRKY72 in Eucalyptus grandis, BrWRKY47 in Brassica rapa, respectively.
from (Fig. 3). The result indicated that *GbWRKY31* was closely related to *ArWRKY6* of *Arabidopsis thaliana* and *TwWRKY* of *Taxus wallichiana*. From the above, *GbWRKY31* belongs to the group IIb of the WRKY families.

**Discussion**

The *GbWRKY31* gene was isolated from ginkgo in this study. The multiple sequence alignment by using bioinformatics analysis software indicated that *GbWRKY31* had high identity with other WRKY genes cloned from other plants. The homologous sequence of WRKY gene among different plants implied that the WRKY gene belongs to the group IIb of the WRKY families and keep a strong conservation during the molecular evolution. The conserved domain motif function further indicated *GbWRKY31* might involve in immune response in plants and might be the key of disease-resistant defense reaction (Peng et al., 2008; Ramiro et al., 2010).

**Conclusion**

*GbWRKY31* with an ORF about 1281bp, encoding 427 amino acids was isolated by RT-PCR from *Ginkgo biloba*. The amino acids of *GbWRKY31* contain a conserved WRKY-motif and a Zinc-finger motif displays extensive homology to WRKY transcription factors from other plants that will enable us to conclude the disease-resistant defense reaction of *G. biloba*. The phylogenetic tree analysis demonstrated the *GbWRKY31* protein belongs to the WRKY IIb group. The *GbWRKY31* was likely to participate in regulating the PR1 promoter activity of disease-resistant defense reaction in ginkgo.

**Conflict of interest statement**

Authors declare that they have no conflict of interest.

**Acknowledgement**

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**References**


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