

Cloning and Expression Analysis of RrG-Beta1 Gene Related to Signal Transduction in *Rosa rugosa*

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Abstract

As an important signal transduction protein, G protein beta subunit gene encoded by oligonucleotides plays an important role in many physiological, biochemical and environmental stresses in plants. In order to understand the action mode of G protein beta subunit gene, this paper cloned a *Wd40* gene related to G protein beta subunit gene, named RrG-beta1, based on the *R. rugosa*—transcriptome data, using *Rosa rugosa* “Zi zhi” as experimental materials. The full length of cDNA of the gene was obtained by RT-PCR and RACE methods. The total length of this gene is 981 bp, and it encodes 326 amino acids. After bioinformatics analysis, the molecular formula $C_{1601}H_{2520}N_{450}O_{486}S_{11}$ was predicted; the relative molecular weight was 36,201.00 Da; the theoretical isoelectric point PI value was 6.71; and its instability index was 30.44. The total average hydrophobic index was -0.847 . In the secondary structure of RrG-beta1 protein, there are 17 α -helix, 131 Random coil, and 141 extended peptide chain. Gene Bank Blast results showed that the amino acid sequence encoded by RrG-beta1 was more than 90% homologous with the beta-like protein of *Rosa chinensis*, *Fragaria*, *Malus*, *Pyrus*, *Prunus*, *arabidopsis* and *tobacco*, so it can be inferred that the RrG-beta1 Gene is guanine nucleotide-binding protein subunit beta-like protein. Fluorescence quantitative expression analysis of RrG-beta1 protein decreased with the development of flower color, and it was speculated that it could exert negative regulation effect on flower color. The leaf expression was highest in the tissue part, so it was inferred that the signal was transmitted through the stoma on the leaf.

Keywords

Rosa rugosa, *G β* , Signal Transduction, Drought Stress, Gene Expression

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1. Introduction

Rosa rugosa is an ornamental shrub of the *Rosaceae* family, it has long history of cultivation, so people call it “flower queen” [1]. The roses were gorgeous and fragrant. Because of its easy cultivation and management, rose has an important role in landscaping and greening [2]. As a functional protein of a stable complex, *Wd40* has a variety of biochemical and cellular biological functions, mainly including signal transduction, RNA processing vesicle transport, cytoskeleton assembly and a variety of biological processes [3]. For example, it can form *MBW* complex with *MYB* and *BHLH* to regulate anthocyanin biosynthesis [4] and can interact with *Rack1* to regulate plant physiology, biochemistry and response to environmental stress [5]. Beta subunit gene refers to a protein that can bind to guanine nucleotides. It is the earliest discovered *Wd40* protein, which is composed of trimer protein and “small *Gi*” protein [6]. Among them, heterologous trimer G protein is composed of three subunits α , β , γ ; the receptor is activated after binding with the signaling molecule [7]. The activated receptor further activates the corresponding G protein, and then delivers to the corresponding effectors at the first level to regulate the physiological functions of plants [8]. *G β* is a typical *Wd40* repeat protein with only known spatial structure [9]. This gene has been cloned from most mammals, but little research has been done in higher plants [10]. At present, only *Zea mays* [11], *Arabidopsis* [12], *Tobacco* [13] and *Oryza sativa* [14] were cloned and analyzed. At present, there are systematic studies on the functions of *G β* protein in mammals and simple eukaryotic cells [15]. However, there are few studies on higher plants. It is not clear whether the signal pathway of *G β* protein in plants is consistent with that in animals and whether there is a protein that plays a role together with *G β* protein.

2. Material and Methods

The experiment was conducted from April 2017 to January 2018 in the flower germplasm resources nursery of Shandong agricultural university and the flower research institute of forestry university.

2.1. Plant Material

The plant materials, Chinese representative *Rosa rugosa* “Zi zhi”, *Rosa rugosa* “Bai zi zhi” Plants were selected from April to May 2017 with robust, stable and pure designs, and the half-open petals of the above three varieties were collected as the experimental materials for gene cloning. The flower petals of the above three varieties were collected in the bud stage, the initial stage, the half stage, the blooming stage and the end of the blooming period as the differential expression test among the varieties; Five flowering stages and root, stem, leaf, pistil, stamen, sepals were collected for tissue differential expression test. All samples were collected directly frozen with liquid nitrogen, and finally stored at -80°C until used.

2.2. Methods

2.2.1. Total RNA Extraction and cDNA Synthesis

According to the instructions of EASY spin plant RNA rapid extraction kit (Aidlab Biotech, Beijing, China), RNA of all tissue parts and total RNA of *Rosa rugosa* “Zi zhi” petals were extracted. Their concentration and purity were determined by ultraviolet spectrophotometer. Meanwhile, their integrity was detected by 1% agarosegel electrophoresis (Thermo Fisher Scientific, Wilmington, Delaware, USA). The first strand of reverse transcription synthesis cDNA was synthesized by RNA reverse transcription and according to the instructions of abm reverse transcription kit (ABM Company, Vancouver, Canada).

2.2.2. PCR Cloning of Anthocyanin Biosynthesis Related Gene

According to the notes of the *R. rugosa*-transcriptome database, 48 *Wd40* genes were isolated and specific primers were designed using Oligo 7.0 software (**Table 1**). The reaction system included 1 μ L cDNA, 1 μ L F1 primer (10 μ mol/L), 1 μ L R1 primer (10 μ mol/L), and 12.5 μ L PCR MIX, with ddH₂O added to a total volume of 25 μ L. The reaction conditions were: 94°C for 5 min; 94°C for 30 s, 53°C for 30 s, and 72°C for 1 min for a total of 35 cycles; and then extension at 72°C for 10 min. Next, 1% agarosegel electrophoresis was used to detect the PCR products. The target PCR fragment was recovered with the Hipure Gel Pure DNA Mini Kit (Magen). The recovered fragment was ligated to the pMD18-T vector and then transformed into *Escherichia coli* DH5 α . The positive clones were selected and sent to BGI for sequencing.

2.2.3. Bioinformatics

Homologous sequence alignment analysis was performed using Blast online provided by NCBI. The open reading frame for *Wd40* gene cDNA was found online, using ORF Finder predict protein secondary structure, using online software Prot-Param and CD-Search was used to analyze the physical and chemical properties of proteins and the prediction of conservative structural domains. Build the phylogenetic tree using MEGA5.0 software.

2.2.4. Expression Analysis of RrG-Beta1 in Different Tissues and Different Flower Developments

Following the instruction of SYBR®Premix ExTaq™ kit by CFX96™ Real-Time System RT-qPCR instrument and referencing to abm reverse transcriptase kit to synthesize cDNA and use it as template for real-time fluorescence quantitative PCR method to detect the expression of *Wd40* gene in 5 developmental stages and 7 tissue sites. According to the sequence information of *Wd40* gene cloned, the primers were designed by using DNAMAN software (**Table 1**). The reaction volume was comprised of 20 μ L containing 10 μ L SYBR®Premix Ex Taq™, 0.4 μ L primer (RrG-beta1-Q-F and RrG-beta1-Q-R) and 1 μ L cDNA, with ddH₂O added to a total volume of 20 μ L. The reaction conditions were as follows: pre-heating at 94°C for 5 min; 39 cycles at 95°C for 10 s, at 60°C for 30 s. Signals were monitored by the Chromo 3 real-time PCR system, finally 30 s at 60°C and 30 s at 95°C for

the melting curve. Each gene was set to repeat three times, and the experimental data were processed by the method of marking. Each gene was assessed with three biological replications. The relative expression levels of the genes were calculated by the $2^{-\Delta\Delta CT}$ method.

2.2.5. Construction of Expression Vector

According to the ORF of RrG-beta1, the corresponding primers RrG-beta1-S-F and RrG-beta1-P-R (Table 1) were designed. After the sequencing validation, selecting right sequence to extract plasmids, the RrG-beta1 was digested by *Spe*I and *Pml*I, and connected to the vector pCAMBIA1304 by Solution-I. The diagram of pCAMBIA1304 was shown in Figure 1.

3. Results and Analysis

3.1. Cloning and Sequence Analysis of RrG-Beta1

A *Wd40* protein was cloned from *R. rugosa* “Zi zhi” petals, named RrG-beta1. The RrG-beta1 gene 3' terminal sequence of 262 bp length by using nested PCR method (Figure 2(a)) and the full length of the gene was 981 bp (Figure 2(b)), and the open reading frame was 717 bp, encoding 326 amino acids. We obtained the cDNA of this gene successfully through RNA reverse transcription. The nucleotide sequences by BLAST and the translated amino acid sequences were compared on NCBI, and it was found that the amino acid sequences encoded by RrG-beta1 were as much as 90% homologous with the beta-like proteins of *Rosa chinensis*, *Fragaria*, *Malus*, *Pyrus* and *Prunus*. Therefore, it can be inferred that the RrG-beta1 gene is guanine nucleic acid binding to the hypoprotein.

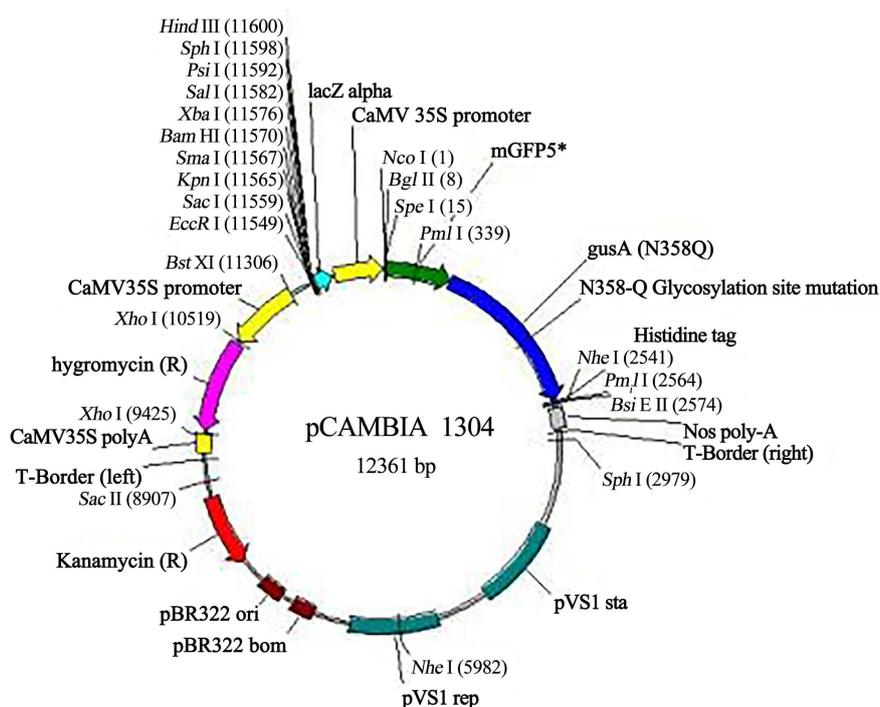


Figure 1. Diagram of pCAMBIA1304.

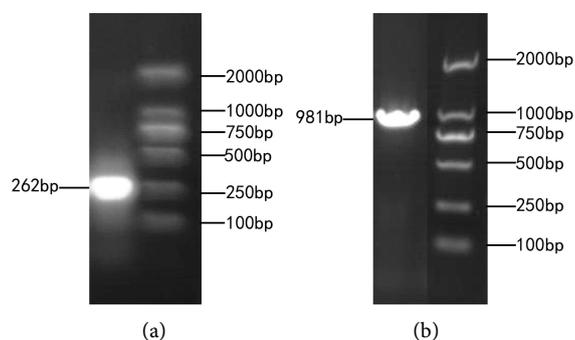


Figure 2. The results of 3'RACE amplification and full-length CDS amplification of the RrG-beta1 gene. (a) 3'RACE amplification product of the RrG-beta1 gene. (b) Full-length CDS amplification product of the RrG-beta1 gene.

Table 1. Primers used in the present study.

Primer name	(5'→3') Nucleotide sequence*	Purpose
β -F	ATGGGTTCCGAAGGCCTC	Intermediate segment amplification
β -R	GAACAATGTGCTTCCATCTGC	
B ₂₆	GACTCGAGTCGACATCGATTTTTTTT TTTTTTTTT	3'RACE PCR for RrG-beta1
β -3'-F	GCAGATGGAAGCACATTGTTCT	Full-length cDNA for RrG-beta1
RrG-beta1-F	ATGGGTTCCGAAGGCCTC	
RrG-beta1	GGGAATTGGCCGCTTCTAG	qRT-PCR for RrG-beta1 and Actin
<i>Actin</i> -F	CACTTAGCACCTTCCAGCAGATGT	
<i>Actin</i> -R	CTACAACAGCAGACCTGAGTTCCT	Expression vector construction for RrG-beta1
RrG-beta1-Q-F	ATGAGGGCCACACCGAC	
RrG-beta1-Q-R	TGAGCTGCCAGAGGATGATG	Expression vector construction for RrG-beta1
RrG-beta1-S-F	<u>ACTAGTATGGGTTCCGAAGGCCTC</u>	
RrG-beta1-P-R	<u>CACGTGCTAGAAGCGCCAATTCCCC</u>	

3.2. Bioinformatics Analysis of RrG-Beta1 Gene

The RrG-beta1 gene encodes 326 amino acids, and the predicted molecular formula is C₁₆₀₁H₂₅₂₀N₄₅₀O₄₈₆S₁₁, the relative molecular weight is 36201.00 Da, and the theoretical isoelectric PI value is 6.71. Among the 326 amino acids coded, 37 basic amino acids (Arg + Lys) and 38 acidic amino acids (Asp + Glu) are included. Its instability index is 30.44, belonging to the unstable protein; The total average hydrophobic index was -0.847, belonging to hydrophilic protein. The secondary structure of RrG-beta1 demonstrates that there are 17 α -helix, 131 random coil, 141 extended peptide chain. The phosphorylation site prediction results reveals that there are 31 Ser phosphorylation sites, 25 Thr phosphorylation sites, and 22 Tyr phosphorylation sites, so we can infer that it may participate in phosphorylation control.

3.3. Construction of Plasmid for Transient Gene Expression Assay

DNAMAN analysis shows that Rrg-beta1 gene is highly homologous to the *Rosa*

chinensis (xp-024172800), genetic homology with *Fragaria* (xp-004299548), *Malus* (xp-008374821), *Pyrus* (xp-009360100), *Prunus* (008240184) are respectively 93.59%, 92.99%, 93.29%. RrG-beta1 gene is 85% homologous to RACK *Phaseolus vulgaris* (ACJ24167), RACK1-like *Morus notabilis* (ANK58717) and GLRACK1 *Glycine max (L.) Merr* (q39836). Predicting the conserved domain of Rrg-beta1 protein amino acid sequences, the results showed that the amino acid sequences of Rrg-beta1 protein had typical *GH* and *WD* dipeptide structures, in which 7 *WD* repeats and 7 *GH* repeats were internal sequences of the protein kinase conserved domain, and their number and location were basically consistent with that soybean, rice and other plants PKC. The N-terminal of the Rrg-beta1 protein is *GH* and the C-terminal is *WG*, and the sequences between the sixth and seventh *GH-WD* core sequences are significantly different from those of other plants, indicating that the protein is significantly different from other homologous proteins (Figure 3).

In order to study the evolutionary relationship between RrG-beta1 protein and other *Wd40* protein, we constructed phylogenetic trees, which showed that RrG-beta1 was closely related to the G-beta families and RACK families, while it was relatively distant from other *Wd40*s in different families (Figure 4).

3.4. Expression Analysis of RrG-Beta1 in Different Tissues and Different Flower Developments

To analyze the specificity of RrG-beta1 gene in different parts and varieties, RT-pcr analysis was used to detect the transcription level of the gene in the root, stem, leaf, petals, pistils, stamens, sepals of the *R. rugosa* “Zi zhi”. Real-time fluorescence quantitative results of gene RrG-beta1 showed (Figure 5): the expression of this gene was significantly high in leaves, followed by the expression in petals, slightly expressed in roots, stems and pistils, and almost not expressed in stamens. The expression of this gene in five different periods of *R. rugosa* “Zi zhi” and *R. rugosa* “Bai zi zhi” was analyzed. The expression of *R. rugosa* “Bai zi zhi” was higher than that of *Rosa rugosa* “Zi zhi” in the bud stage, the initial stage, the blooming stage and the end of the blooming period. Overall, the gene expression of both varieties was the highest at the end of the flowering stage (Figure 6).

3.5. Construction of Plasmid for Transient Gene Expression Assay

After the expression vector pCAMBIA1304 was cut with the restriction endonuclease with *SpeI* and *PmlI* (Figure 4), the RrG-beta1 gene was connected with the vector successfully by cutting through the solutionI to form a pCAMBIA1304-RrG-beta1 (Figure 7). Finally, we obtain the agrobacterium vector and intend to transfer the recombinant plasmid RrG-beta1-pCAMBIA1304 to *Arabidopsis thaliana* to verify its function.

4. Discussion

In this study, RrG-beta1 was cloned from *Rosa rugosa*. G protein beta subunit

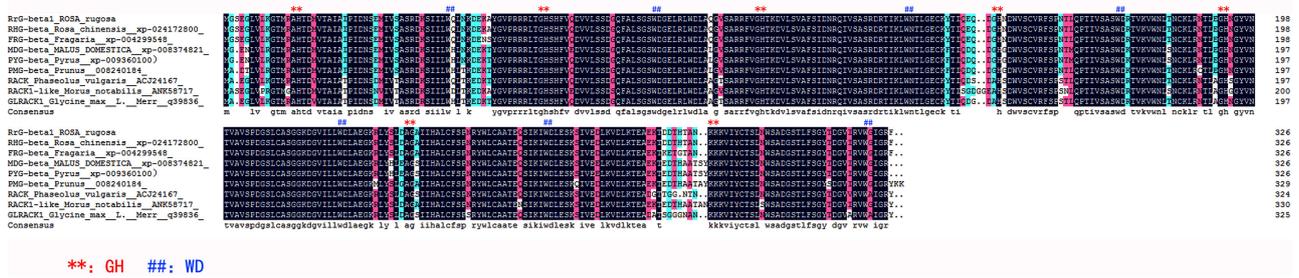


Figure 3. Sequence alignment between RrG-beta1 protein and other homologous proteins (## represented GH loci; ** represented WD loci).

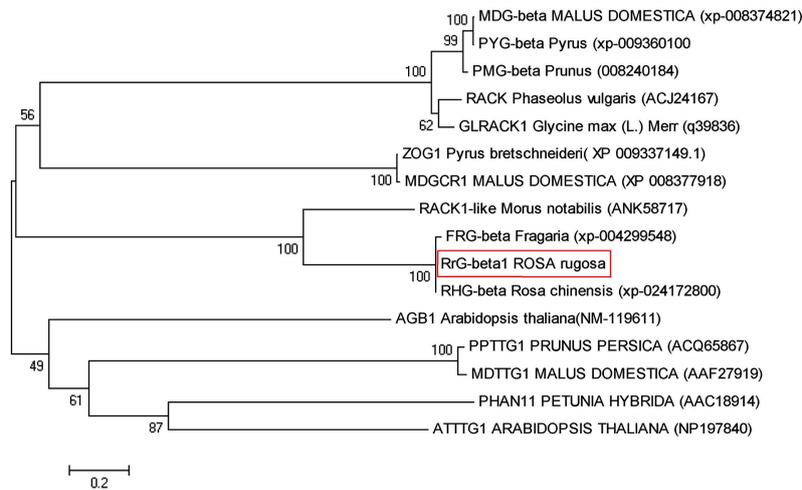


Figure 4. Phylogenetic tree of RrG-beta1 and Wd40 members from other plant species. The tree was constructed by neighbor-joining method using MEGA5.0 software. Branch numbers represent as percentage of bootstrap values in 1000 sampling replicates and scale indicates branch lengths.

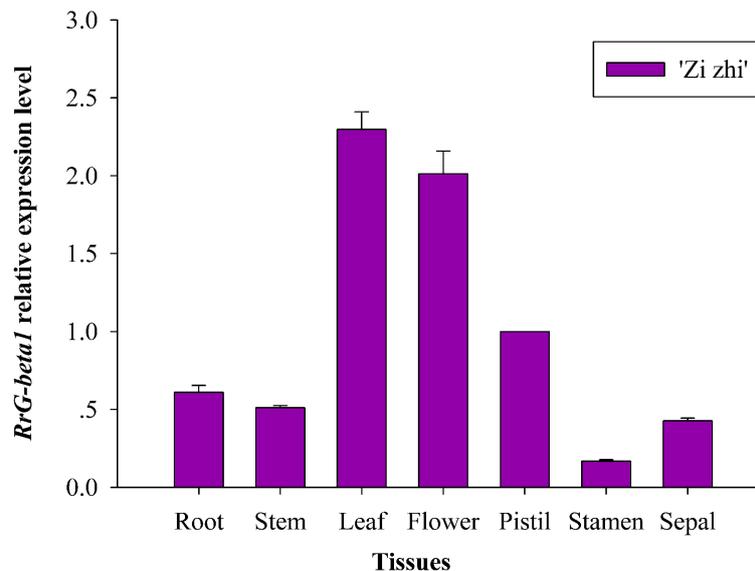


Figure 5. The relative expression of RrG-beta1 in seven different tissues of *R. rugosa* "Zi zhi". RrGAPDH was used as the internal control. The experiment was repeated three times with similar results.

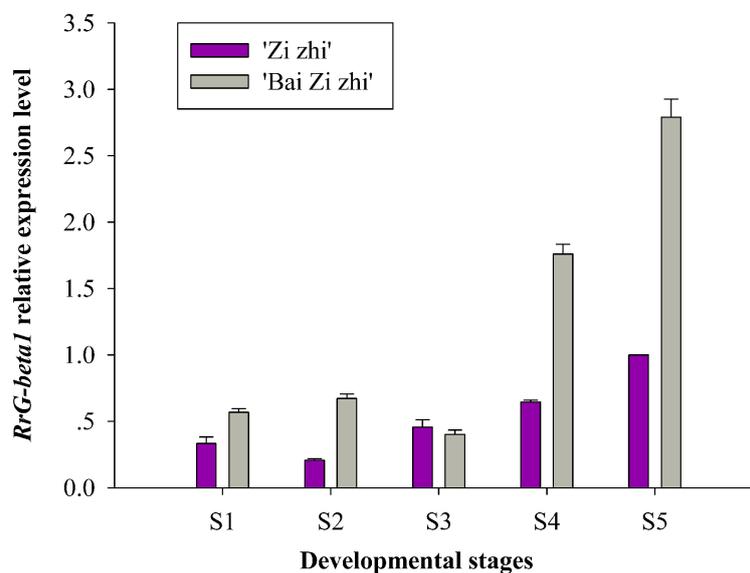


Figure 6. The temporal and spatial expression patterns of RrG-beta1. The relative expressions of the RrGT1 gene in five flowering stages of *R. rugosa* “Zizhi”, and *R. rugosa* “Bai zi zhi”.

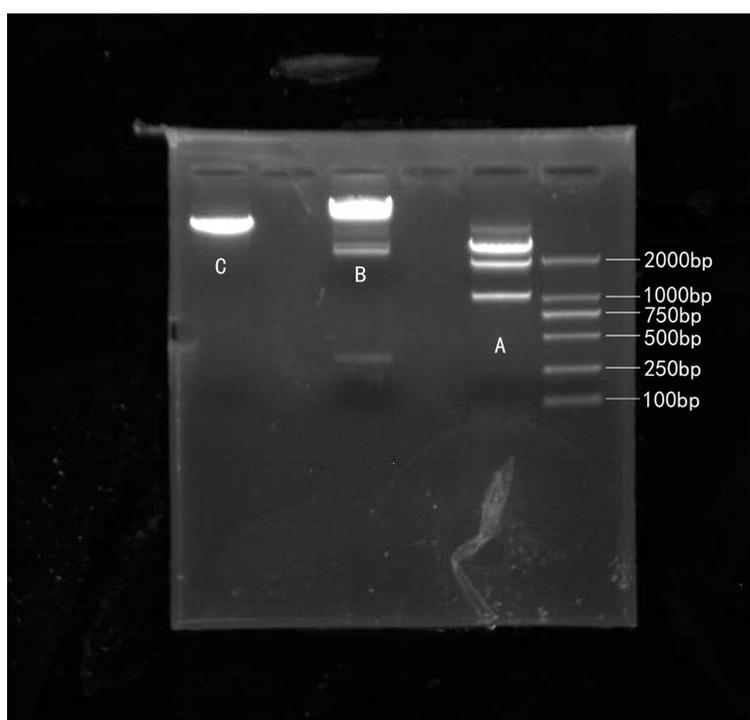


Figure 7. Identification of recombinant expression vector by double enzyme (A represented the target band of RrG-beta1, B represented the target band of pCAMBIA1304, C represented pCAMBIA1304 with no enzyme).

gene is a multifunctional protein that regulates signal transduction, mRNA splicing, and constitutes the cytoskeleton [16]. Through bioinformatics analysis, it was found that RrG-beta1 protein belongs to the $G\beta$. $G\beta$ has two conservative domains, one is a relatively conservative N-terminal formation of α -helix, which

can be nearly parallel and interact with the N-terminal helix of $G\beta$ [17]. Two Cys residues at N terminal can be cross-linked chemically; the other domain consists of seven membrane-like propeller structures composed of seven sets of 40 to 43 repeated fragments of amino acid residues, which contains specific aspartic acid and glycine [18]. It may participate in the regulation of the β and γ combination and adapt to multiple conformation variations of subunits [19]. Therefore, it is concluded that RrG-beta1 protein may be related to cell division and growth development of plant.

The RrG-beta1 amino acid sequences cloned from *Rosa* were compared with the amino acid sequences of other species, which shows that RrG-beta1 is more than 90% similar to G protein in *Rosa chinensis* and *Fragaria*, and more than 85% similar to RACK1 protein in *Arabidopsis*, *Morus notabilis*, etc. Previous studies have shown that RACK1 and G protein genes are highly homologous, and they play a synergistic role in gene function. $G\beta$ protein usually is used as the joint and enzyme regulator of transmembrane signal transduction [20], while RACK1, as the link of information transmission, transmembrane signal transduction into intracellular signal and transmission of genes from intracellular signal molecules to various regulatory functions [21]. It provides the basis and platform for plant cell division, growth and development, hormone regulation and signal transduction.

The results of quantitative PCR showed that the expression of RrG-beta1 gene was higher in *R. rugosa* "Bai zi zhi" than in *R. rugosa* "Zi zhi". It has been found that the trimer G protein of RrG-beta1 gene is the physiological regulatory protein of photosensitive pigments. When photosensitive pigments are activated by light, they first activate G protein and then regulate gene expression through a series of intermediates [22]. Through previous studies, it can stimulate the synthesis and effect efficiency of apple photosensitive element by adjusting light to greatly increase the content of anthocyanin in apple fruits, which proves that as the medium between light and anthocyanin, photosensitive element is indirectly related to the synthesis of plant anthocyanin. The fluorescence quantitative results suggested that RrG-beta1 protein might inhibit the synthesis and regulation of photosensitizer, resulting in the reduction of anthocyanin synthesis. In the expression of different tissues of *R. rugosa* "Zi zhi", the highest expression is leaves, followed by flowers, pistils and roots. Chen *et al.* used the split ubiquitin technique to study the mechanism of the response of *Arabidopsis* $G\beta$ and RACK1 to the drought stress signal [23]. All of them can sense the drought stress signal and transmit the signal to the stoma on the leaf, it can control the stoma closure and by stoma conductivity to reduce water loss [24]. Ma li geng *et al.* first confirmed that G protein was involved in the germination and pollen tube growth of a variety of pollen through a series of pharmacological experiments [25]. The expression level of RrG-beta1 gene in flowers, leaves, pistils and roots is consistent with the results of previous studies, which can be inferred that $G\beta$ gene and RACK1 also play a role in cell signal transduction in *Rosa rugosa*.

5. Conclusion

With the development of animal cell signal transduction research, the study of plant cell signal transduction has been paid more and more attention. At present, only G protein related to cell signal transduction has been cloned in the model plants of *Arabidopsis thaliana* and tobacco, and has not been found in flower varieties. In this study, we cloned RrG-beta1 and analyzed its expression, which was beneficial to analyzing the regulation mechanism of plant cell signaling; what is more, it also provided some important information to research drought resistant in *Rosa rugosa*.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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