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Expression Vector Construction and Genetic Transformation of *Rosa rugosa* β-l,3-Glucanase Gene (*RrGlu*)

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Abstract

In order to lay a foundation for researching the function of *Rosa rugose* (*R. rugosa*) *RrGlu* gene, the *RrGlu* gene was amplified from the styles of *R. rugosa* "Tanghong", a gene expression vector named PBI121-*RrGlu* was constructed and the vector was introduced into tobacco with the agrobacterium-mediated method. PCR results showed that the *RrGlu* gene was integrated into the tobacco genome.

Keywords

Rosa rugose, β-l,3-Glucanase Gene, Expression Vector Construction, Genetic Transformation

1. Introduction

R. rugosa is a Chinese traditional famous flower. The short florescence and uniform color limit its application in landscaping. The hybridization of the *R. rugosa* with the *R. hybrid*, which flowers throughout the year and has diverse colors, can greatly improve its ornamental trails. However, the incompatibility of interspecific hybridization is caused by pollen tube stopping growth in style, which seriously inhibited the process of *R. rugosa* bleeding. It has been reported that the large amount of callose deposition at the top of the pollen tube and the intercellular space of the style channel may be the critical factors hindering the growth of the pollen tubes [1] [2] [3]. Furthermore, it is important to note that a similar phenomenon appeared in the interspecific hybridization of the Actinidia, *Lilium*, and iridaceae plants, as well as the self-pollination of the Rosaceae, Sola-

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naceae, and Theaceae plants [4] [5] [6] [7]. Therefore, callose may play a very important role in plant pollination incompatibility.

Callose is *β*-l,3-glucan. Its synthesis and hydrolysis are catalyzed by *β*-l,3-glucan synthase (Cals) and *β*-l,3-glucanase (Glu) [8] [9]. Previous studies show that *β*-l,3-glucanase inhibits fungal growth by degrading *β*-l,3-glucanase in the fungal cell wall. Hence, *β*-l,3-glucanase is an important pathogenesis related protein [10] [11] [12]. According to our previous work, we propose that it is very likely that the *Glu* gene regulates plant pollination compatibility. However, current studies about the *Glu* gene mainly focus on genetic engineering of plan disease resistance [13] [14]. There is no report about its role in pollination compatibility. Previously, we cloned the full length of the *RrGlu* gene (GenBank accession number: KU144821) and analyzed its bioinformatics characteristics [15]. In this study, we established the over expression vector of the *RrGlu* gene and transferred it into tobacco. Our study provides a basis for investigating the function of the *R. rugosa RrGlu* gene in the future.

2. Material and Methods

2.1. Material

2.1.1. Plant Material

R. rugosa "Tanghong" was cultivated in the rose germplasm nursery of Shandong Agricultural University. *N. tabacum* "K326" was stored in our laboratory.

2.1.2. Plasmids and Strains

E. coli DH5*α* and *A. tumefaciens* GV3101 strains were purchased from Beijing Tiandz Genetech Ltd. The vector PBI121 was constructed by our laboratory.

2.1.3. Reagent

Restriction endonucleases, gel extraction kits, and PCR reagents were all purchased from Dalian Takara Biotechnology Inc. EASY spin plant RNA extraction kits were purchased from Aidlab Biotechnologies Co., Ltd. Kanamycin and Kefotaxime were purchased from Beijing Solarbio Science & Technology Co., Ltd.

2.2. Methods

2.2.1. Cloning of the *R. rugosa RrGlu* Gene

The total RNA in styles of *R. rugosa* "Tanghong" was extracted and used for cDNA synthesis. Specific primers

F (5'-GCTCTAGAATGTCTAAATGCAATTCTTCAG-3') and

R (5'-CGGGATCCATTGAAATTGATAGGGTATTTTGG-3') were designed according to the *R. rugosa RrGlu* gene sequence published on GenBank (KU144821) and used to amplify *R. rugosa RrGlu* gene. Primer F contains *Xba* I restriction site, while primer R contains *BamH* I restriction site. The reaction system included 1 μL cDNA, 1 μL F primer (10 μmol/L), 1 μL R primer (10 μmol/L), and 12.5 μL PCR MIX, with ddH_2O added to a total volume of 25 μL. The reaction conditions were: 94˚C for 3 min; 94˚C for 30 s, 55˚C for 30 s, and 72˚C for 30 s for a total of 36 cycles; and then extension at 72˚C for 10 min. Next, 1% agarose

gel electrophoresis was used to detect the PCR products. The target PCR fragment was recovered with the MiniBEST Agarose Gel DNA Extraction Kit Ver. 3.0 (TaKaRa). The recovered fragment was ligated to the pMD18-T vector and then transformed into *E. coli* DH5a. The positive clones were selected and sent to BGI for sequencing.

2.2.2. Construction of the Expression Vector PBI121-*RrGlu*

The *RrGlu* fragment with *Bamh* I and *Xba* I ends was cloned into a PBI121 vector. The obtained plasmid was transferred into *E. coli* DH5a. After the restriction ends were confirmed by the double digestion method, the recombinant PBI121-*RrGlu* plasmid was transferred into GV3101 strain using the freezing-thawing method. PCR was used to detect the construction.

2.2.3. Tobacco Genetic Transformation

Aseptic tobacco seedling leaves were cut into 0.5 cm × 0.5 cm pieces, which were cultured in MS medium supplemented with 3 $mg \cdot L^{-1}$ 6-BA and 0.6 $mg \cdot L^{-1}$ NAA under light for 2 days, followed by transgenic infection for 8 min (OD_{600} = 0.5). Then, the leaves were cultured in the above medium in dark for two days, followed by screening culture in the above medium supplemented with 100 $mg \cdot L^{-1}$ Kanamycin and 400 $mg \cdot L^{-1}$ Kefotaxime. Resistant callus and buds were induced. When the resistant buds in tobacco callus were 2 cm in length, these buds were transferred into the rooting MS medium supplemented with 0.6 $mg \cdot L^{-1}$ NAA, 100 $mg \cdot L^{-1}$ Kanamycin and 400 $mg \cdot L^{-1}$ Kefotaxime.

2.2.4. Molecular Detection of Transgenic Plants

The genomic DNA of wild type and transgenic tobacco leaves was extracted. Primers F and R were used for PCR amplification to detect whether the *RrGlu* gene is successfully integrated with tobacco genome. RNA of wild type and transgenic tobacco leaves was extracted and used for cDNA synthesis. Primers F and R were used for RT-PCR amplification to detect whether *RrGlu* is expressed.

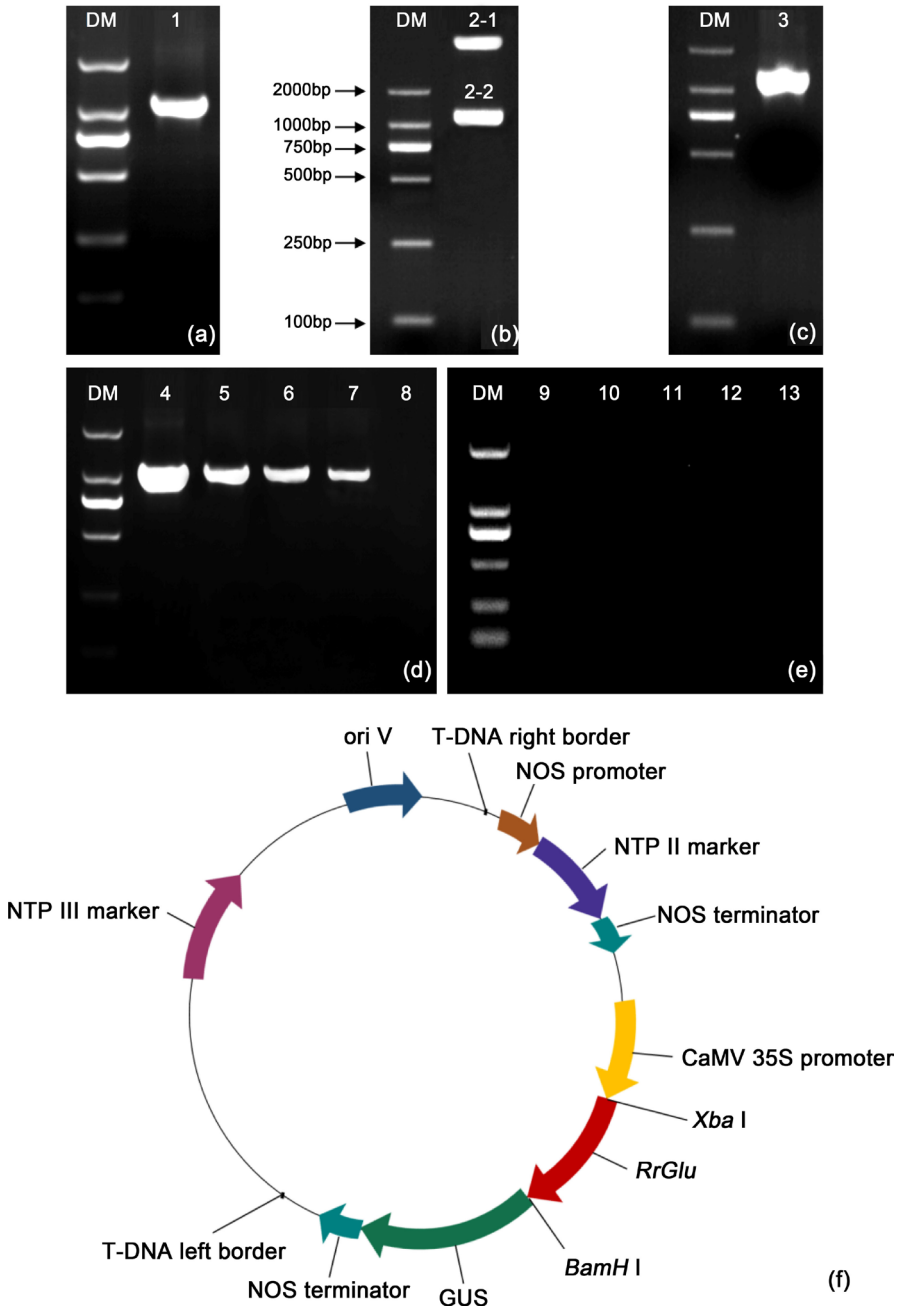
3. Results and Analysis

3.1. Cloning of the *R. rugosa RrGlu* Gene

F and R primers with the restriction endonuclease ends were used for PCR amplification. The obtained PCR products were about 1100 bp (**Figure 1(a)**) which was the same size as expected.

3.2. Construction of the Expression Vector PBI121-*RrGlu*

The size of digested PBI121-*RrGlu* fragment was the same as expected (**Figure 1(b)**), so the expression vector was successfully constructed. The recombinant PBI121-*RrGlu* was transferred into GV3101 strain. PCR detection recognized a specific fragment (**Figure 1(c)**), suggesting that the PBI121-*RrGlu* expression vector was successfully transferred into GV3101 strain. The sequencing data also indicated that the *RrGlu* fragment was integrated into the downstream of 35S promoter with a forward direction. The structure of expression vector PBI121-*RrGlu* was showed in **Figure 1(f)**.



DM. DNA marker; 1. Product of *RrGlu* amplification; 2-1, 2-2. Digestion of the recombinant plasmid; 3. Amplification with PBI121-*RrGlu*; 4, 5, 6, 7. PCR product of transgenic tobaccos; 8.PCR product of wild type tobacco; 9, 10, 11, 12. RT-PCR product of transgenic tobaccos; 13. RT-PCR product of wild type tobacco.

Figure 1. (a) PCR amplification of *RrGlu*; (b) Confirmation of the recombinant plasmid PBI121-*RrGlu* with digestion; (c) Confirmation of the recombinant plasmid PBI121-*RrGlu* by PCR amplification; (d) Confirmation of transgenic tobaccos with PCR; (e) Confirmation of transgenic tobaccos with RT-PCR; (f) Structure of expression vector PBI121-*RrGlu*.

3.3. Tobacco Genetic Transformation and Molecular Detection of Transgenic Plants

6 tobacco plants which grew best were selected. Leaf genomic DNA was ex-

tracted and detected using PCR. The results show that the specific band (about 1100 bp) was detected in six plants, while it was undetected in the control plant (**Figure 1(d)**). It indicated that the *RrGlu* gene was integrated into the tobacco genome. RNA was extracted from the leaves of wild type and transgenic tobacco and used for cDNA synthesis. Primers F and R were used for RT-PCR detection. The results showed that there was no *RrGlu* specific band in the transgenic tobacco (**Figure 1(e)**), suggesting that the *RrGlu* gene could not be expressed in the leaves of transgenic tobacco plants.

4. Discussion

Previous studies show that β-l,3-glucanase inhibits fungal growth by degrading β-l,3-glucanase in the fungal cell wall. Hence, β-l,3-glucanase is an important pathogenesis related protein. At the same time, β-l,3-glucanase gene is involved in the development of a variety of plant growth processes, such as microsporogenesis, pollen tubes growing, fruit ripening and seed germination [16] [17] [18] [19]. According to our previous work, we propose that it is very likely that the large amount of callose deposition at the top of the *R. rugosa* pollen tubes and the intercellular space of the *R. hybrid* style channel may be the critical factors hindering the growth of the *R. rugosa* pollen tubes [20]. Therefore, we established the over expression vector of the *RrGlu* gene and transferred it into tobacco. Our study provides a basis for investigating the function of the *R. rugosa RrGlu* gene in the future.

Under normal conditions, the expression of β-1,3-glucanase gene is pretty low in plants. When the plant is induced by pathological or relative conditions, the enzyme accumulates rapidly and its expression activity is obviously enhanced [21] [22]. Moreover, β-l,3-glucanase is unevenly distributed in a plant. It is barely detectable in young leaves [23] [24]. Our preliminary studies support this concept. The expression level of *RrGlu* gene was extremely low in normal *R. rugosa* leaves, but it is strongly expressed in style, especially pollinated styles. It suggests that the *RrGlu* gene may be related to *R. rugosa* pollination compatibility. In this study, the *RrGlu* gene had been integrated into tobacco genome, but its expression was undetectable in transgenic tobacco leaves. It is possible that the *RrGlu* gene can be transcribed normally, but it cannot be normally expressed in tobacco young leaves. It may be primarily expressed in tobacco styles. However, the tobacco plants are still young, and the theory will be tested in the future during the flowering period.

In this study, the gene expression vector named PBI121-*RrGlu* was constructed and the vector was introduced into tobacco. PCR results showed that the *RrGlu* gene was integrated into the tobacco genome, which provided the basis for further research on the function of *RrGlu* gene.

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