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Functional Characterization of UDP-Glucose Pyrophosphorylase Gene from Jute (Corchorus Capsularis L.) and Its Role in Cellulose Synthesis in Arabidopsis Thaliana

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Abstract
In this study, we subcloned the full-length cDNA of the UDP-glucose pyrophosphorylase gene of Corchorus capsularis into expression vector pCAMBIA 1301. Arabidopsis were transformed by floral-dip method, PCR and southern hybridization results indicate exogenous gene was integrated into the Arabidopsis genome. Overexpression of CcUGPase gene revealed increased height and more rapid growth rate in transgenic Arabidopsis compared with control lines. Importantly, the transgenic Arabidopsis have more cellulose content than control lines, while the lignin content remained unchanged. The results indicate that jute UGPase gene participates in cellulose biosynthesis in plants, which provides an important basis for the application of the UGPase gene in the improvement of jute fiber quality.

1. Introduction

UDP-glucose pyrophosphorylase (UGPase) is an important regulatory enzyme in glucose metabolism of plants, anamal and fungi, which was first cloned in yeast cells in 1953 (Munch et al, 1953; Daran et al, 1995). It catalyze the glucose-1-phosphate (Glc-1-P) reaction with Uridine triphosphate (UTP) to form uridine diphosphate glucose (UDPG) and pyrophosphate (PPi), which is the key precursor involve in cellulose, hemicellulose, pectin and glycolipids, glycoprotein synthesis metabolism (Eimert et al, 1996; Kleczkowski et al, 2004). UDPG can also be converted to ADPG by the coupled reactions of UGP and cytosolic AGPase in starch synthesis (Eimert et al, 1996). Uridine diphosphate glucose pyrophosphorylase (UDP-glucose pyrophosphorylase, UGPase) is a precursor of catalytic cellulose-uridine diphosphate glucose (UDP-glucose, UDPG) synthesis. Studies have shown that the UGPase gene is closely related to cellulose biosynthesis

(Qing et al, 2011).

UGPase plays important roles in carbohydrate metabolism and secondary cell wall biosynthesis (Spychalla et al, 1994; Kleczkowski et al, 2010). To date, UGPase cDNAs

have been cloned in potato (Katsube et al, 1991), banana (Pua et al, 2000), Barley (Eimert et al, 1996), Rice (Abe et al, 2002; Chen et al, 2007), Melon (Dai et al, 2006), Aspen (Meng et al, 2007) and Cotton (Qing et al, 2011). Current studies showed UGPase gene closely associated with biosynthesis of cellulose, the cellulose content has been shown to increase after transfer the bacterial UGPase gene into tobacco, while lignin content was unaffected (Liu et al, 2002). Transfer of the cotton UGPase gene increased cellulose content in Arabidopsis thaliana (Qing et al, 2011). Furthermore, overexpression of UGPase in S. zooepidemicus resulted in a slight alteration in virulence and a reduction in the cell envelope hyaluronic acid yield. Jute (Corchorus Capsularis L; Family, Tiliaceae) species, also known as Luo Ma, is an annual bast fiber crop. Jute is one of the most important fiber crops in the world, with the planting area and yield almost equal to that of cotton. Jute fibers exhibit a characteristically high luster, good moisture absorption performance, rapid water loss capacity and easy degradation. Consequently, jute has become known as the "gold fiber" in recent years. As a result of the continuous efforts of breeders, jute fiber production has been greatly improved; however, jute fiber cell wall lignification and other limiting factors render the short, coarse fibers suitable only for the production of rope and sacking. These characteristics seriously restrict the field application of jute fiber in the textile industry.

The quality of jute fiber is very poor mainly due to the low cellulose content (jute: 57%-60%, ramie: 65%-75%, cotton: 87%-90%) (Lan et al, 2009). So cloning and functional analysis of the jute UGPase gene will provide new insights into potential improvements in the quality of jute fibers and cellulose biosynthesis. So, the purpose of this study is to analyse the function of UGPase gene in cellulose biosynthesis, evaluated its critical role in cellulose synthesis following over expression in Arabidopsis.

2. Materials and Methods

2.1. Plant Material and Treatments

Arabidopsis thaliana (ecotype Columbia) was provided by the Rice Research Institute, Fujian Academy of Agriculture Sciences (China). Intact seeds were selected and subjected to vernalization for 2 days at 4ºC. All seeds were cultured in nutrient soil with a light/dark photoperiod of 18 h light/6 h dark, at 24 and 18ºC and humidity maintained at 70%. The flowers of seedlings were used as transformation recipients.

2.2. Plasmid Construction

The open reading frame (ORF) of CcUGPase was amplified using the following primers: Sense primer, 5'-GAAGATCTCGTCTCCAGTCCACACCAAATCC-3' and antisense, 5'-GGGTAACCGAAACTCTCCCGCAACATACACAA-3' (BglII and BstEII restriction endonuclease sites are shown in underlined red letters). The amplified PCR product was digested with BglII and BstEII and inserted into a binary vector pCAMBIA 1301 containing a hygromycin phosphotransferase (hph) gene, for expression under the control of the CaMV35S promoter (Fig 1). Sequence analysis confirmed the correct insertion of the UGPase gene into the vector.

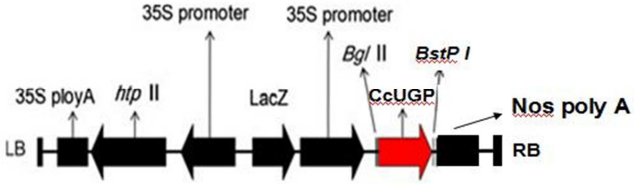


Fig. 1. *Outline of the pCAMBIA 1301 trnsformation vector with CcUGP under the control of CaMV 35S promoter.*

2.3. Transformation of Arabidopsis Thaliana and Screening

The constructed vector containing the CcUGPase gene was introduced into *Agrobacterium tumefaciens* strain EHA105 by the freeze–thaw method (Holsters et al, 1978). Agrobacterium-mediated transformation of Arabidopsis plants was performed by the floral-dip method (Clough et al, 1998). Transgenic lines were obtained by selection on MS culture medium containing hygromycin (30 mg/L) and confirmed by PCR amplification using a gene-specific primer: 1304-F (5'-TGTTCTCTCCAAATGAAATGAACT -3') and a vector-specific primer: 1304-R (5'-AATCAGAGCCAGTATCCCCG-3') (Zhang G et al, 2013). The PCR product was detected in 1% agarose gel electrophoresis. Chose positive plants confirmed by PCR for southern bolt detection, according to the transformation vector plasmid [PCAMBIA1301], choose three single restriction sites, respectively is: BSTE Ⅱ, BG Ⅲ and NcoI, 5-10 μg genomic DNA was digested overnight. Hygromycin probe labeled with alkaline phosphatase Labeling Kit (ROCHE company). The hygromycin probe primer (R: 5 '-CATACTTGAGACCAGTGTTT-3', F: 5 '-CCGACCTTAACTAGCTCAAT-3'), the results was collected use the gel imaging system Fluor Chem SP 50mm f11.4 lens.

2.4. Phenotype Analysis of Transgenic Arabidopsis Thaliana

The morphology and growth rate of transgenic and control plants was analyzed. Each group comprised five strains and the analysis was repeated three times. The results were analyzed by SPSS LSD variance analysis.

2.5. Determination of Lignin and Cellulose Content

Lignin and cellulose content was determined using the method described (Huntley et al, 2003; Updegraff et al,1969; Zhang G et al, 2013).

3. Results and Discussion

3.1. PCR and Southern Hybridization Detection of Transgenic Arabidopsis

In the screening of transgenic Arabidopsis, To ascertain the expression of CcUGPase gene in transgenic seedlings, total RNA was isolated and RT-PCR was performed using primers specific (primer1: 5'-CAACTCCTGATGATCCAGCTG-3', primer2: 5'-GGCTTGTGGAAGCTGATGCA

CTC-3') for UGPase gene transcripts. The PCR-positive (Fig 2) plants were selected as putative transgenic Arabidopsis. Genomic DNA was isolated from Arabidopsis leaves of randomly selected three PCR-posive plants and used for Southern blot analysis with hygromycin specific probe further confirmed the success of the transgenic Arabidopsis. The results (Fig 3) indicated that all the selected plants have T-DNA integrated in Arabidopsis genome and contain one or two insertion sites of the tansgene, this will remain stable in Genetic process of transgenic offsprings. No hybridization bands were detected in the non-transgene plants.

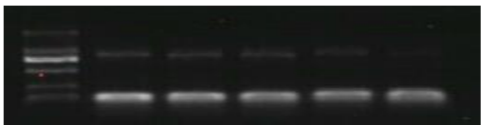


Fig. 2. *The result of PCR amplification M: DL2000.*

Marker. 1, 2, 3, 4, 5: Represent different samples.

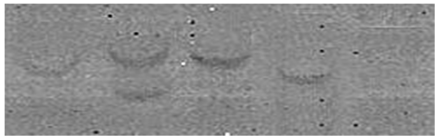
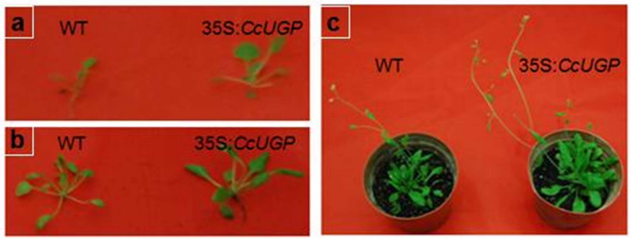


Fig. 3. *The result of Southern hybridization.*

1: Positive control of Pcambia1301 vector. 2: Digested by BSTE Ⅱ enzyme. 3: Digested by BG Ⅲ enzyme. 4: Digested by NcoI enzyme. 5: The negative control

3.2. Phenotype Observation, Lignin and Cellulose Contents of Transgenic Lines

Significant phenotypic differences were observed between with CcUGPase gene transgenic lines and the wild-type Arabidopsis at different stages, with transgenic Arabidopsis exhibiting a faster growth rate (Fig 4 a, b) and increased height (Fig 4 c). More detailed analysis of the differences between the transgenic and non-transgenic Arabidopsis lines were carried out by determining the internode distance and plant height at 60 d (Table 1). The results indicated a significant increase in plant height of the transgenic lines compared with the wild-type, although no difference in the internode distance was detected. In contrast to previous studies (Zhang G, et al,2013), the aim of this study is to find out the specific pathway of *UGPase* gene involvement in *A. thaliana* in the future. The data indicate that CcUGPase gene involved in plant growth and development (Qing et al, 2011). This is consistent with previous studies on the insertion of an exogenous UGPase gene into tobacco, which resulted in increased growth rate and plant height (Coleman et al, 2006; Zrenner et al, 1993). These findings suggest that UGPase plays a key role in the strength of sink tissues and the increased growth rate resulted in greater production of fibers compared with non-transgenic plants (Coleman et al, 2006). Some research report that UDP-glucose pyrophosphorylase is not rate limiting for sucrose/starch and cell wall synthesis, but is essential in Arabidopsis (Meng et al, 2009; Park et al, 2010).



a Phenotype of 15-day-old plants. b Phenotype of 25-day-old plants.

Fig. 4. *Morphology of wild and transgenic Arabidopsis.*

They displayed faster rate than wild type. c transgenic plants (right) were much higher than wild type (left) after 60 days.

Cellulose is a polysaccharide composed of glucose and widely used in paper making and textile industry. Measurement of the cellulose and lignin contents (Table 1) of transgenic and control plants at 60 d revealed no significant differences in the lignin contents (Wild-type: 17.2%; Transgenic line 1: 17.7%; Transgenic line 2: 16.8; Transgenic line 3: 17.2), while the cellulose content was markedly higher in the transgenic Arabidopsis lines than that in control (Table 1). These results are consistent with those of previous studies (Qing et al, 2011). UGPase catalyzes the conversion of uridine triphosphate into uridine diphosphate glucose, which serves as the glucosyl donor involved in cellulose synthesis. Jute fiber content is much lower than cotton, ramie (Lan et al, 2005; Zhang et al, 2011), if we can greatly improve the jute fiber content, jute is likely to be like cotton application in textile industry. Therefore, it can be speculated that CcUGPase affects plant cellulose synthesis based on the results obtained in this study.

Table 1. *Morphology analysis of wild-type and transgenic Arabidopsis.*

Lines	Internode distance (cm)	Height (cm)	Cellulose (%)	Lignin (%)
Control	3.3±0.26	21.3±0.7	20.8±0.9	17.2±0.5
Transgenic line 1	3.3±0.29 a	23.6±1.2 b	24.7±1.1 a	17.7±1.0 a
Transgenic line 2	3.3±0.29 a	25.0±1.3 a	25.5±1.9 a	16.8±1.4 a
Transgenic line 3	3.2±0.19 a	23.1±1.2 b	25.3±1.0 a	17.2±0.4 a

Each value represents mean of five replicates ± SD. Means were compared using ANOVA.

The different letter after data within a column represents significant difference at 5% probability level.

4. Conclusions

In summary, over expression vector PCAMBIA1301 was construction, we obtain a large number of transgenic *A. thaliana* using floral-dip method. PCR and Southern hybridization results indicate exogenous gene was integrated into the Arabidopsis genome. Over-expression of the CcUGPase gene in Arabidopsis resulted a faster growth rate and increased cellulose content compared with the non-transgenic control plants. These results suggest that the CcUGPase gene plays a key role in cellulose synthesis. This information will provide the basis of further investigations aimed at improving jute crop fiber quality.

Abbreviations

UDP	Uridine diphosphate
UGPase	UDP-glucose pyrophosphorylase
UDPG	Uridine diphosphate glucose
PPi	Pyrophosphate
UTP	Uridine triphosphate
AGPase	ADP-glucose pyrophosphorylase

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