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Full Length Article

Construction of Rice *OsRSR1* Gene Interference Vector and the Gene Expression of Enzymes Involved in Endosperm Starch Synthesis

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Abstract

This study was conducted to explore the method of rapidly constructing the Os*RSR1* gene interference expression vector pBWA (V) HS-*OsRSR1* and analyze the effect of *RSR1* gene on the regulation of gene expression in key enzymes of rice starch synthesis. Based on the principle of Golden Gate technology, the *OsRSR1*-RNAi vector was constructed quickly and transferred into rice through Agrobacterium-mediated method to obtain genetic transformation plants with interference expression. We measured the transcriptional expression quantity of the endosperm *OsRSR1* gene and genes of enzymes involved in starch synthesis in the transgenic plants and the wild type control plants at different stages of the grain filling. Our results show that the positive conversion rate of the interference expression vector constructed in this experiment was 75%, and the degree of gene interference was about 60%. In the process of grain filling, the transcriptional expression quantity of *OsRSR1*gene in the wild type control and transgenic plant grains showed a V shaped change trend, and the low point appeared in 20 days after heading. However, the transcriptional expression quantity of the key enzyme genes *GBSSI, AGPL2, SSI, SBEIIb* and *ISAI* of starch synthesis all showed a single peak curve change trend, and the peak appeared in 20 days after the heading. Our results suggest that *OsRSR1* plays a negative role in expression of the enzyme genes *GBSSI, AGPL2, SSI, SBEIIb* and *ISAI* of starch synthesis in grains and should be further investigated for quality breeding. © 2018 Friends Science Publishers

Keywords: Rice; *OsRSR1* gene; RNA interference vector; Starch synthesis; Amylose and total starch

Introduction

The biosynthesis and accumulation of rice starch were mainly carried out in the chloroplast or amyloplast, and the reaction was completed by a series of starch synthesis key enzymes, including ADPG pyrophosphorylase (AGPase), granular binding starch synthase (GBSS), soluble starch synthase (SSs), starch branching enzymes (BEs), starch debranching enzymes (DBEs), starch phosphorylase (PHO) and other key enzymes (Georgelis *et al*., 2009). Among them, ADPG was the rate-limiting enzyme in the synthesis of endosperm starch (Boehlein *et al*., 2008), which directly determined the synthesis rate and the accumulation level of starch. GBSS catalyzed the synthesis of amylose. The main role of SSs, BEs and DBEs was to complete the synthesis of amylopectin together. The relationship between the activity of the starch synthase and the synthesis and accumulation of the endosperm starch has been reported at home and abroad (Cai *et al*., 1998; Shu *et al*., 1998; Fang *et al*., 2002; Hirose and Terao, 2004; Nakamura *et al*., 2005; Tetlow *et al*., 2008; Wang *et al*., 2013).

The expression of structural genes is regulated by many internal and external factors, and the transcription factor is one of them. AP2/EREBP transcription factors play an important role in plant growth, development, and various physiological and biochemical reactions, adversity, stress and other signal transduction. *RSR1* (Rice Starch Regulator1) belongs to the transcription factor in the AP2/EREBP family. The expression of *OsRSR1* gene is negatively correlated with the expression of some starch synthase genes. The lack of *OsRSR1* leads to an increase in the expression quantity of starch synthesis genes in the seeds. However, the function of *OsRSR1* in regulation of key gene expression in starch synthesis is not very clear.

To clarify the effect of transcription factor OsRSR1 on gene expression of key enzymes in starch synthesis and starch content, we constructed *OsRSR1*-RNAi vector according to the principle of RNAi technology to inhibit the transcriptional expression of *OsRSR1* gene in rice. This vector was used to convert the callus of low amylose content line Dongnong 1124 seed through Agrobacterium-mediated method, and the anti Hyg plants with transformed *OsRSR1* gene were obtained. And the rice genetic transformation system was established. Finally, through the rigorous series testing, the *OsRSR1* gene RNA interference transgenic rice lines was obtained. This transgenic line and the non-

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transgenic control Dongnong 1124 were selected. Pot experiments were conducted to compare and analyze the change of the transcriptional expression quantity of genes *GBSSI*, *AGPL2*, *SSI*, *SBEIIb*, *ISAI* and other key enzymes genes of starch synthesis and the change of amylose and total starch content in grains at different stages of grain filling. Here, our results provide a theoretical basis for elucidating the molecular regulation mechanism formed by starch quality.

Materials and Methods

Materials

The experiment was carried out at the Rice Laboratory of College of Agriculture of Northeast Agricultural University in 2014~2017 years. The tested varieties were low amylose content (7.5%) line Dong Nong 1124 and Dongnong 1124 calli, which were used for the vector construction of the *OsRSR1*-RNAi and the establishment of the genetic transformation system.

Vector and Reagent

The *Escherichia coli* strain DH5α, Agrobacterium tumefaciens strain EHA105 and pCAMBIA-1301 vector were preserved in the laboratory. pBWA (V) HS vector was purchased from Biorun Company. Restriction enzyme (*Bsa*I), T4 DNA ligase, kanamycin (Kan), carbenicillin (Carb), and hygromycin (Hyg) were purchased from TaKaRa Company. The standard molecular weight Trans2k DNA Marker was purchased from TransGen Biotech Company.

Culture Medium

Preculture medium: MS+2.0 $mg \cdot L^{-1}$ 2, 4-D; Co culture medium: MS+2.0 $mg \cdot L^{-1}$ 2, 4-D +100 $mg \cdot L^{-1}$ acetosyringone; Selection medium: MS+2.0 $mg \cdot L^{-1}$ 2, 4-D +50 $mg \cdot L^{-1}$ Hyg+250 $mg \cdot L^{-1}$Carb; Differential medium: MS+2.0 $mg \cdot L^{-1}$ 6-BA+0.25 $mg \cdot L^{-1}$ NAA+0.1 $mg \cdot L^{-1}$; Rooting medium: 1/2MS.

Methods

Primer design and PCR reaction system: Based on the reported mRNA sequence of the rice *RSR1* gene (GenBank login number is AY685117) (Fu and Xue, 2010), the synthetic primers were designed. The two target fragments of *OsRSR1-1* and *OsRSR1-2* are the same sequence of *OsRSR1* gene, and the sequence of primers is as follows: *OsRSR1-1*-S: 5′cagtGGTCTCAtttgtacttcttgccaaggaattg3′, *OsRSR1-1*-A:5′cagtGGTCTCTcaggtgcagggcgtacgacagggc3′, *OsRSR1-2*-S:5′cagtGGTCTCAgggctgcagggcgtacgacagggc3′, *OsRSR1-2*-A:5′cagtGGTCTCTagagtacttcttgccaaggaattg3′. Partial primers of the Loop: Loop-S 5′cagtGGTCTCAcctgcaggtctagtttttct3′, Loop-A:5′cagtGGTCTCTgcccgggctctgtaactatc3′. The PCR amplification of the three fragments took the Dongnong 1124 DNA as a template. The reaction system was DNA polymerase (5U/μL),0.25 μL; 10×PCR Buffer (Mg^{2+}Plus), 5 μL; dNTPs (each 2.5 m*M*), 2 μL; DNA template, 1 μL; Upstream primer(10 μM), 1 μL; Downstream primer (10 μM), 1 μL; Amplification procedure: predegeneration at 94℃ for 5 min, degeneration at 94℃ for 30s, annealing at 50~58℃ for 1 min, extension at 72℃ for 1 min, total extension at 72℃ for 10 min, heat preservation at 16℃, 30 cycles. After agarose gel electrophoresis, the PCR products were recovered by AxyPrep DNA gel extraction kit.

Construction of Interference Vector pBWA (V) HS-RSR1

The reference (Yang *et al*., 2014) method of recovery product enzyme cutting and vector connection of three target segments: The following reagents were added to the 0.5 mL centrifuge tube in turn: T4 ligase Buffer, 2 μL; BsaI, 1 μL; T4 ligase, 1 μL; pBWA (V) HS, 4 μL; Recovery fragment, 4 μL; Total volume 20 μL, The reaction temperature was 37℃, and the reaction time was one hour. The competent Escherichia coli was transformed by freezing and thawing methods, the positive recombinant expression plasmids were screened and the sequencing was carried out.

Agrobacterium Transformation

The positive recombinant silent plasmid was imported into the Agrobacterium tumefaciens EHA105 and transformed by liquid nitrogen freezing and thawing method. After 48h cultivation on the YEB agar medium containing 50 $mg \cdot L^{-1}$ Hyg, white plaque appeared. After picking single colony shaking bacteria, the plasmid was extracted and taken as the template. Then the upstream and downstream primers of specific primer *OsRSR1-1* were used to make the PCR amplification for it. The PCR product was detected by electrophoresis on 1% agarose. After the transformed Agrobacterium tumefaciens EHA105 colony was propagated, it was preserved at -80℃.

The Establishment of Rice Genetic Transformation System

The yellowish, compact and granuliform embryogenic callus was picked and put in a small glass bottle. The callus was soaked for 20 min by Agrobacterium whose concentration had been adjusted and it was shaken a few times during this period. The callus was taken out and the bacteria solution was sucked out with filter paper, and then the callus was blown in the upper clean bench and transferred to the co culture medium. Then it was darkly cultured for 3 days at 25℃. After co culture, the callus was taken out and washed with sterile water once, and then it was soaked in the sterile water of 400ppm carbenicillin and 200ppm cephalosporin for 30 min.

The callus was then taken out on the filter paper and blown in the super clean bench (more than 5 h). After drying, the callus was transferred to the screening medium. It was screened 2 times, and 2 weeks each time. The resistant callus with a diameter of 1-2 mm with good growth state, compact structure and light yellow was selected and then transferred to the predifferential medium. After it was darkly cultured for one week at 25°C, it was transferred to the differential medium. After it was cultured for 3-4 weeks at 28°C by light, it started to differentiate seedlings. The seedlings of 3-4 cm were transferred into the rooting medium and screened by Hyg (50 mg/mL) antibiotic root. After they were cultured for 3-4 weeks at 28°C by light and were long to the top of the tube, the sealing film was removed. After 2-3 days, they were removed from the rooting medium (Zhang *et al*., 2013). Then the seedlings with developed roots were selected and the medium on the root was washed, and finally they were planted into the soil, so as to obtain the complete positive plants and obtain the T_0 generation seeds. Then in the same way, the Hyg screening was carried out, and the seeds of T1 and T2 generation were finally obtained.

Detection of Transgenic Rice

The genomic DNA of transgenic rice was used as the template for PCR amplification, screening and identifying the positive plants. At the same time, the rice without *OsRSR1* gene was taken as the negative control. The Trizol method was used to extract the total RNA of the positive plant identified by PCR, and the RT-PCR detection was carried out. The transcriptional expression quantity of the key enzyme gene of starch synthesis was measured by the fluorescence quantitative PCR method. Jarvis (Jarvis and Walker, 1993) and other methods were used to determine the apparent amylose content of the endosperm at each grain filling period. According to the modified sulfuric acid anthrone detection method of Zhaofang He (He, 1985), the total starch content in every grain filling stage of endosperm was determined. The samples were repeatedly measured for three times to calculate the average value.

Data Analysis

Data statistics and analysis were carried out by Microsoft Excel and DPS v7.55 version software.

Results

Construction of *OsRSR1*-RNAi Interference Vector

The construction process of RNA interference vector (*OsRSR1*-RNAi) of *OsRSR1* was shown in Fig. 1. The RNA interference fragment in the *OsRSR1* gene obtained by PCR amplification was cloned on the vector by the RT-PCR method. Through several steps such as PCR product recovery and enzyme cutting connection, 2 *OsRSR1* interference fragments were simultaneously connected to the two sides of the Loop fragment in a reverse complementary manner. The interference vector fragment was connected with the pBWA (V) HS vector complementary *Bsa*I enzyme cutting site. In the process of *OsRSR1*-RNAi vector transcriptional expression, the two target genes sequence was the same. The *OsRSR1* interference fragment formed the hairpin structure together with the Loop fragment in the vector in the reverse complementary manner, triggering the RNA interference effect.

PCR Amplification of Target Gene *OsRSR1*

Dongnong 1124 genome DNA was taken as the template. The two pairs of primers: OsRSR1-1-S and OsRSR1-1-A, and OsRSR1-2-*S* and OsRSR1-2-A were taken as the primers. The pCAMBIA-1301 plasmid was taken as the template, and the Loop-S and Loop-A were taken as the primers to make the PCR amplification. In the cDNA coding region of the gene, two 242 bp amplified bands and one 200 bp amplified band were amplified, which was consistent with the expected results (Fig. 2).

Construction Process of *OsRSR1*-RNAi Interference Vector

Three amplified products were recovered in the same system (the recovery products were marked as: rDNAClC3). After purification, they were connected with the pBWA (V) HS vector with the BsaI enzyme cutting. After the target gene fragment and Loop were recovered, they were directly mixed with the pBWA (V) HS plasmid. The BsaI endonuclease and T4 DNA ligase were added into the recovery tube at the same time. After one step enzyme cutting and connection synchronization reaction, the two target fragments were connected with the left and right sides of the loop by the reverse complementation. The target gene fragment could be simultaneously cloned in a reverse complementary way to pBWA (V) HS to form the hpRNA structural expression vector. The connected vector was transformed into the Escherichia coli DH5α competent cell, and the positive clone was identified through the Escherichia coli plaque PCR amplification. The transformed *Escherichia coli* DH5α was taken as the template, OsRSR1-1-S and Loop-A were used as primers for PCR amplification. The PCR amplified products were separated by 1% agarose gel electrophoresis. The size of the amplified fragments was the same as the predicted size 684 bp (Fig. 3), indicating that the *OsRSR1* fragments had been connected with the pBWA (V) HS expression vector. The *Escherichia coli* bacteria solution corresponding to the four positive bands was taken, and then 100 μL bacteria solution was taken for sequencing. The rest 400 μL bacteria solution was inoculated to the 5 mL LB medium containing kanamycin resistance. Then the bacteria were shaken at 37°C, and the sequencing of bacteria solution was carried out. One tube of bacteria solution with corresponding correct sequencing was taken to extract the *Escherichia coli* plasmid.

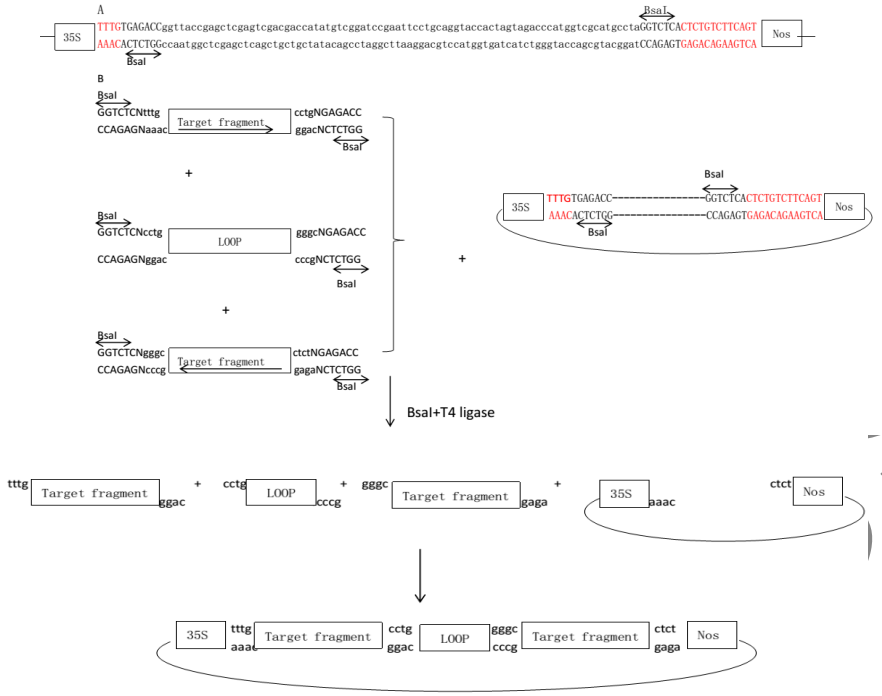

Fig. 1: The construct of *OsRSR1*-RNAi

The Introduction of Plant Expression Vector pBWA (V) HS-*OsRSR1* into Agrobacterium Tumefaciens EHA105

The primers OsRSR1-1-S and OsRSR1-2-A were used to make the PCR reaction for the recombinant plant expression vector pBWA (V) HS-*OsRSR1* transferred to Agrobacterium. Through the amplification, a fragment of 684 bp was obtained, as shown in Fig. 4, indicating that the recombinant plasmid has been transferred into Agrobacterium tumefaciens EHA105.

The Acquisition of *OsRSR1* RNAi Transgenic Rice Plants

The constructed pBWA (V) HS-*OsRSR1*i vector plasmid was extracted, and then the agrobacterium-mediated method was used to transform the constructed plasmid into the callus of rice. The obtained rice callus was screened, differentiated and made the rooting culture by hygromycin 50 mg/L to obtain the transgenic rice plants. After transplanting, the plants that were not genetically transformed were taken as the negative control, and the plasmid containing the target gene was taken as the positive control. The genomic DNA extracted from the leaves of rice transgenic plants and wild type control plants was used as templates. Hyg-S and Hyg-A primers were used to make the PCR amplification. The partial amplification results were shown in Fig. 5.

As shown in Fig. 5, no specific bands of 280 bp were detected in negative control, but clear specific bands were amplified on the positive control and most of the transgenic plants. After Hyg screening, the T_2 generation pure line seed was finally obtained.

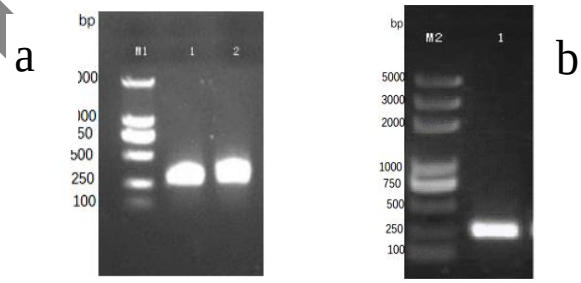

Fig. 2: (a) PCR products of *OsRSR1-1*, *OsRSR1-2*. M1, DL2000; 1, Fragment of *OsRSR1-1*; 2, Fragment of *OsRSR1-2*. (**b**) PCR products of loop.M2, DL5000; 1, Fragment of loop

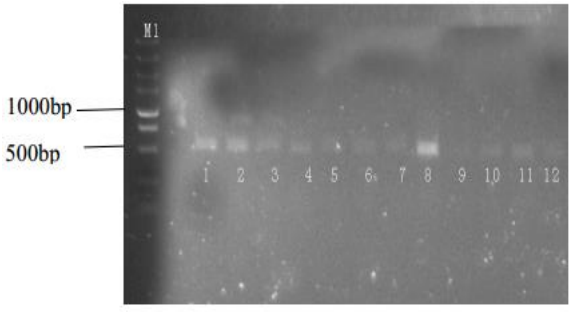

Fig. 3: PCR analysis of *OsRSR1*-RNAi vector
M1, DL5000; the second lane, negative control; 1-12, PCR products of *E. coli* clones

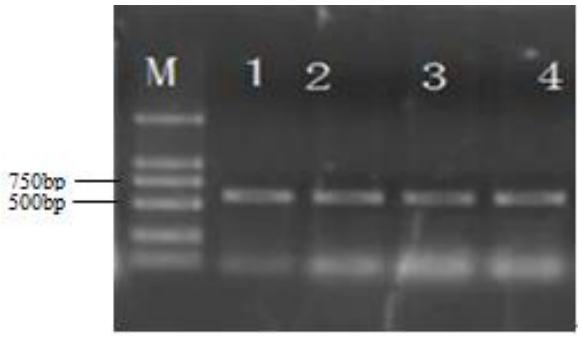

Fig. 4: PCR analysis of bacterial clones
M, DL2000; 1-4, positive Agrobacterium clones

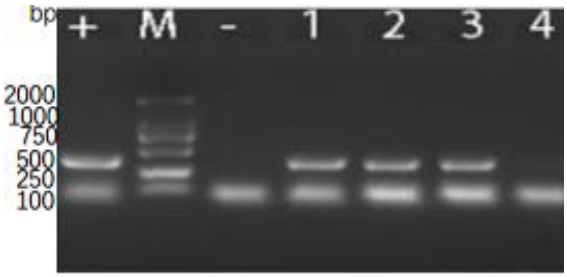

Fig. 5: PCR detection of transformed rice lines
M, DL2000; +, positive control; -, untransformed plantlet; 1-4, transformed plantlets

A total of 55 Dongnong 1124 transgenic plants were obtained in this experiment. Through the above PCR detection methods, 40 positive genetic transformation plants with target genes were obtained, and the positive rate was 75%, indicating that the positive plant acquisition rate of genetic transformation method constructed in this experiment was very high.

Effect of RNA Interference on the Transcriptional Expression Quantity of *OsRSR1* Gene in Transgenic Rice

In order to test the RNA interference effect of *OsRSR1* gene, the transcriptional expression quantity of *OsRSR1* gene in wild type control plants and positive transgenic plants with target gene was detected by fluorescence PCR. The results are listed in Table 1.

It was shown in Table 1 that in the process of grain filling, the transcriptional expression quantity of *OsRSR1* gene in the wild type control and transgenic plant seeds was a V-shaped change trend. That was, after flowering, with the grain filling process, it descended first, and then rose gradually after the low point, and the low point appeared in 20 days after heading. As far as the gene expression quantity was concerned, the *OsRSR1* gene transcriptional expression quantity of positive transgenic plants at different stages of grain filling was significantly lower than that of the wild type control plants, and the decrease was 52.6%~62.4%, indicating that RNA interference had significantly inhibited the transcriptional expression of *RSR1* gene. In this experiment, the *OsRSR1*-RNAi transgenic rice material was successfully obtained, which could be used as the research material for the regulation of downstream structural genes by target gene interference.

Effect of *OsRSR1* Gene Expression Interference on the Gene Transcriptional Expression quantity of enzymes involving in starch synthesis of Grain

The comparative results of the transcriptional expression quantity of partial isoform gene of enzymes involving in starch synthesis of grain of the wild type and transgenic rice plants at different stages of grain filling were shown in Table 2.

According to Table 2, in the grain filling process, the transcriptional expression quantity of key enzyme genes *GBSSI*, *AGPL2*, *SSI*, *SBEIIb* and *ISAI* of starch synthesis in wild type control and transgenic plant grains showed a change trend of the single peak curve. That was, after flowering, it rose gradually with the process of grain filling, and then decreased gradually after the peak, and the peak appeared in the 20 days after heading. In terms of the gene expression size, the transcriptional expression quantity of key enzyme genes *GBSSI*, *AGPL2*, *SSI*, *SBEIIb* and *ISAI* of starch synthesis in the grains of positive transgenic plants at different stages of grain filling was significantly higher than the wild type control plants. The increased range was different depending on the gene and grain filling period. The increased range of *GBSSI* gene was up to 47.47%~66.80%, and the increased range of *SBEIIb* gene was up to 55.50%~123.60%. The increased range of the two genes in the early stage of grain filling was obviously greater than that in the late filling stage.

The increased range of *AGPL2* gene was up to 50.10%~93.32%, and the increased range in the late grain filling stage was greater than the early stage of grain filling. The increased range of *SSI* gene was up to 50.70%~69.60%, and the increased range of *ISAI* gene was up to 53.80%~59.78%. The increased range of the two genes was not greatly changed during the whole grain filling process. It was concluded that inhibiting the transcriptional expression of *OsRSR1*gene could significantly increase the transcriptional expression quantity of starch synthesis related enzyme genes in grains, and the increased range varied with different genes and different filling periods.

Effect of *OsRSR1* Gene Expression Interference on Starch Content

The results of the effect of *OsRSR1* gene expression interference on the starch content of rice were listed in Table 3. From Table 3, it could be seen that the content of amylose and total starch in grain and polished rice of positive transgenic plants at different stages of grain filling was significantly higher than those of wild type control plants.

Table 1: Analysis of *OsRSR1* gene expression of Wild type(WT) and RNAi lines (*OsRSR1*-RNAi)

Variety	Days after heading (d)							
	10		20		30		40	
	expression quantity	the decline range (%)	expression quantity	the decline range (%)	expression quantity	the decline range (%)	expression quantity	the decline range (%)
Wild Type	1.000a	0.0	0.064a	0.0	0.232a	0.0	0.400a	0.0
Transgenic line	0.376b	62.4	0.029b	54.7	0.110b	52.6	0.152b	62.0

Table 2: Comparation of gene expression in *OsRSR1*-RNAi lines and wild type plants

Gene	Variety	Days after heading (d)							
		10		20		30		40	
		expression quantity	the increased range (%)	expression quantity	the increased range (%)	expression quantity	the increased range (%)	expression quantity	the increased range (%)
GBSS1	Wild Type	1.000c	0.0	11.593c	0.0	8.868c	0.0	5.374c	0.0
	Transgenic line	1.668a	66.80	18.051a	55.71	13.807a	55.69	7.925a	47.47
AGPL2	Wild Type	1.000c	0.0	2.461c	0.0	1.182c	0.0	0.987c	0.0
	Transgenic line	1.501a	50.10	4.529a	84.03	2.285a	93.32	1.853a	87.84
SSI	Wild Type	1.000c	0.0	2.439c	0.0	1.897c	0.0	1.452c	0.0
	Transgenic line	1.685a	68.50	3.675a	50.70	3.010a	58.70	2.463a	69.60
SBEIIb	Wild Type	1.000c	0.0	2.623c	0.0	2.364c	0.0	2.289c	0.0
	Transgenic line	2.236a	123.60	5.865a	123.50	4.286a	81.30	3.559a	55.50
ISAI	Wild Type	1.000c	0.0	4.282c	0.0	2.591c	0.0	2.001c	0.0
	Transgenic line	1.589a	58.90	6.842a	59.78	3.985a	53.80	3.129a	56.37

Table 3: Starch content in *OsRSR1*-RNAi lines and wild type plants at filling stage (%)

Starch type	Variety	Days after heading (d)				
		10	20	30	40	Missed rice
amylose content (%)	Wild Type	2.91b	6.04b	6.67b	7.06b	7.49b
	Transgenic line	3.73a	7.59a	7.89a	8.31a	8.61a
	the increased range (%)	28.18	25.66	18.29	17.71	14.95
Total starch content (%)	Wild Type	42.84b	58.14b	64.16b	66.71b	67.08b
	Transgenic line	44.51a	60.06a	65.96a	67.94a	68.26a
	the increased range (%)	3.90	3.30	2.81	1.84	1.76

The increased range of amylose and total starch content was up to 14.95%~28.18% and 1.76%~3.90% respectively, and the increased range gradually decreased with the grain filling process. It showed that the down regulation of *OsRSR1* gene transcriptional expression quantity could significantly promote the synthesis and accumulation of amylose and total starch in grains.

Discussion

Construction of RNA Interference Vector for *OsRSR1* Gene

For the construction of RNA interference vectors, many investigators have carried out a lot of research and have created many different construction methods (Xu *et al*., 2010; Manamohan *et al*., 2013; Yan *et al*., 2013; Huo *et al*., 2016). The Golden Gate cloning method uses the IIs type restriction enzyme, which has the characteristics of cutting outside the recognition site, and has no specific requirements for the sequence of the cutting site. The cohesive end produced after the enzyme cutting can be made up of any basic group, which makes the DNA fragment do not have the original enzyme cutting site after splicing. The process of enzyme cutting and connection can be carried out simultaneously, and multiple DNA fragments can be connected simultaneously. In this experiment, the fragment with the length of 242 bp was selected as the target gene according to the technique principle of this method. At the same time, the two ends of the primers had *Bsa*I enzyme cutting site and the length of the loop was 200bp. After the three fragments were cloned, the *Bsa*I enzyme and T4 DNA ligase were added to the cloned fragments and vector at the same time, and the hpRNA expression vector was constructed by one step enzyme cutting-connection synchronous reaction. In this experiment, the three recovered fragments could complete the construction of RNAi vector only through a *BsaI* enzyme cutting and connection synchronization reaction in the same centrifuge tube, without the need of the electrophoresis recovery process of the enzyme fragments, and the construction efficiency was high without background interference. The vector pBWA (V) HS used in this experiment was the plant binary expression vector. The constructed pBWA (V) HS-RNAi expression vector could be used directly for the transformation of rice callus mediated by Agrobacterium tumefaciens.

In this experiment, the Golden Gate cloning technology was applied to construct the hpRNA interference vector of *OsRSR1* transcription factor successfully, and the interference vector was introduced into rice seed callus by Agrobacterium tumefaciens transformation, and the transgenic plants were obtained. The transformation rate was up to 75%. The monitoring results of *OsRSR1* gene transcriptional expression quantity also showed that the transcriptional expression quantity of *OsRSR1*gene of positive transgenic plants at different stages of grain filling was significantly lower than the wild type control plants. The decreased range was up to 52.6%~62.4%, indicating that the transcriptional expression quantity of *RSR1* gene in the RNA interference vector constructed by Golden Gate cloning method has been significantly inhibited, providing reliable materials for analyzing the transcriptional expression of downstream structural genes.

Regulation of Starch Synthesis in Grain

GBSSI, AGPL2, SSI, SBEIIb, ISAI and so on are key enzymes involved in starch biosynthesis of grains. The functions of each synthetase in the process of starch biosynthesis are different, for example, AGP is mainly related to the synthesis rate of total starch and the final starch synthetic amount (Tetlow, 2006), the activity of pyrophosphorylase has a significant or extremely significant positive correlation with the accumulation of grain starch (Nakamura *et al*., 2005), SSS and SBE are mainly related to the synthesis of amylopectin, finally affecting the composition and structure of starch in caryopsis (Hirose and Terao, 2004; Tetlow *et al*., 2008), GBSS mainly controls the synthesis of amylose (Sasaki *et al*., 2000; Yan *et al*., 2013; Javed Iqbal Wattoo *et al*., 2015), which is the key enzyme for controlling the ratio of amylose to total starch in rice grains (Wang *et al*., 2013), GBSS I is the key enzyme in the synthesis of endosperm amylose (Fang *et al*., 2002; Oh *et al*., 2018). And each enzyme plays a different role in the process of starch synthesis. However, the starch biosynthesis is accomplished with the participation of many above enzymes. The gene expression quantity, enzyme activity and phenotypic expression also change synchronously, that is, the increase of gene expression quantity will increase the activity of the enzyme, and the increase of the activity of the enzyme will enhance the performance of the trait.

Conclusion

Our results show that the Golden Gate cloning method is highly effective in RNA interference vector construction in *OsRSR1* function analysis in starch synthesis. Further data analysis suggest that *OsRSR1* plays a negative role in expression of the enzyme genes *GBSSI, AGPL2, SSI, SBEIIb* and *ISAI* of starch synthesis in grains. Therefore, transformed application of *OsRSR1* gene in rice yield and quality will achieve high yield and good quality coordination effect, which is named as “one gene and many effects”.

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