African Journal of Biotechnology Vol. 11(30), pp. 7628-7637, 12 April, 2012
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB11.3807
ISSN 1684–5315 © 2012 Academic Journals

Full Length Research Paper

Increasing the amylose content of maize through silencing of *sbe2a* genes

Shuyan Guan[1], Yiyong Ma[2], Huijing Liu[1], Siyan Liu[1], Guangna Liu[1], Lina Zhao[1] and Piwu Wang[2]*

[1]College of Life Sciences, Jilin Agricultural University, Changchun 130118, China.
[2]Agronomy College of Jilin Agricultural University, Changchun 130118, China.

Accepted 23 March, 2012

Improved amylose content in maize has been achieved by reducing the starch branching enzyme (SBE) activity via transgenic maize inbred line transformed by a high-efficient RNAi expression vector, which may provide the foundation for maize quality improvement. The sense and anti-sense fragments of maize SBE gene *sbe2a* were cloned by reverse transcript PCR and high efficient RNAi expression vector was constructed based on plant expression plasmid pCAMBIA1301. Then the reconstruct was introduced into maize inbred line Tie7922 by pollen tube pathway transformation. Four transgenic plants were obtained. The integration of interest gene *sbe2a* into maize genome has been confirmed by PCR amplification and Southern hybridization. The *sbe2a* transcription was suppressed obviously by the analyses of RT-PCR, SBE activity and amylase content on the four transgenic plants. The SBE activity was significantly less than that of wild type maize, and was at most reduced by 77.9%; the amylose content was at most increased by 87.8%. In conclusion, RNAi expression vector pRSBE2a containing *sbe2a* gene was successfully constructed. Through genetic transformation, RNAi technique efficiently silences endogenous *sbe2a* gene to reduce the SBE activity, and high-amylose maize lines are obtained.

Key words: Maize, starch branching enzyme gene *sbe2a*, RNA interference, genetic transformation.

INTRODUCTION

Corn starch, a main component of kernel, includes different molecular structures of amylose and amylopectin. Corn amylose, which is characterized by a high degree of polymerization and good film forming, is far superior to other amylose in the areas of support films, food, medical treatments, textiles, papermaking, packaging, petroleum, environmental protection, optical fibers, printed circuit boards, and electronic chips (Visser and Jacobsen, 1993). Corn high amylase is the best material for manufacture of photodissociative plastics and could potentially help to control serious "white pollution" (Smith et al., 1997).

Currently, the amylose used for industry mainly comes from corn. Amylase for Chinese market mainly imports from the United States. Amylose extracted from normal maize is costly; therefore, breeding high-amylose maize varieties can significantly expand the applications of corn starch and promote development of the corn industry, improving economic benefits (Casey et al., 2000). Starches are produced by a synthetic process that is regulated by a series of enzymes. Starch branching enzyme (SBE) is a key enzyme in the process of starch biosynthesis, forming the branched structure by catalysis of glucose monomer binding through α-1,6 bonds (Denver et al., 2001). SBE is composed of two families; SBE(A) and SBE(B), and corn SBE has three isozymes; SBEI, SBEIIb and SBEIIa. SBEI and SBEIIb are mainly present in the endosperm and SBEIIa in the embryo, endosperm, leaves, and other nutritive tissues. Together, these three enzymes participate in the synthesis of amylopectin (Nunes et al., 2006; Qiao et al., 2007). Corn SBEIIa can directly participate in the synthesis of short chains of amylopectin, and has much higher activity than

*Corresponding author. E-mail: peiwuw@yahoo.com.cn. Tel: 0431-84532908.

Abbreviations: SBE, Starch branching enzyme.

SBEIIb, indicating that the functions of SBEIIa and SBEIIb cannot be complementary (Yandeau-Nelson et al., 2011). In addition, deficiency of the SBEIIa isoform resulted in lower kernel yield (Blauth et al., 2001; Yandeau-Nelson et al., 2011).

RNA interference (RNAi) is an effective and specific method of gene silencing, capable of posttranscriptionally regulating gene expression through the activity of double-stranded RNA molecules. This method has been widely applied in the area of unknown genetic function in both plants and animals, and in the area of crop improvement of rice (Qiao et al., 2007), maize, soybean (Nunes et al., 2006), barley (Schweizer et al., 2000), wheat (Sestili et al., 2010), patato and cotton (Sunilkumar et al., 2006), especially in the improvement of corn high amylose quantity and quality by *sbe* gene cloning and amylopectin suppression. Sestili et al. (2010) used RNAi of *sbe2a* to increase the amylose content of durum wheat. However, there are only few reports of corn starch composition changes as a result of RNAi of *sbe2a*, the gene for SBEIIa enzyme (Koga et al., 2006; Sestili et al., 2010).

The present study was to clone corn SBE gene, *sbe2a*, and construct an efficient RNAi vector pRSBE2a. The pRSBE2a construct was transferred into inbred maize line Tie7922 by pollen tube pathway transformation, in order to achieve the inhibition of the SBE gene to produce high amylose. This study also investigated the genetic effect of gene interference, as well as potential applications of RNAi technology in crop improvement.

MATERIALS AND METHODS

Materials

Waxy corn inbred line "W1" was a gift from Prof. Yu-Lan Wang, Jilin Agricultural University; recipient maize inbred line Tie7922, modified pCAMBIA1301 plasmid were preserved by the laboratory. Concer^{TM} plant RNA extract kit and reverse transcript kit were purchased from Invitrogen China (Shanghai); pMD18-T vector, PCR amplification kit, restriction enzymes, *Escerichia coli* DH5α from TaKaRa (China Dalian); T4 DNA ligase and DNA markers from Promega China (Beijing); nylon membranes, digoxin (DIG) labeling and testing kit from Roche China (Shanghai); amylose and amylopectin standard samples were purchased from Sigma Company; other reagents of analytic purity were obtained from China.

Cloning and sequencing of corn sbe2a

The 562 bp of the total coding sequence (CDS) of *sbe2a* was used for designing primers (implying that the entire coding sequence was larger than 562 bp) (NCBI GenBank accession no. U65948), using Primer Premier 5.0 software (Premier, Canada). Primer sequences were as follows (restriction sites such as *Sac* I, *Xho* I, *Xba* I and *BamH* I are underlined): P1 upstream primer: 5′-TCTTG AGCTC ATAGG CGAGA ATCCC ACAT-3′ and downstream primer: 5′-TAACC TCGAG CGTGT AAAGA TACGG ATGGA-3′; P2 upstream primer: 5′-TTTGT CGACC GTGT AAAGA TACGG ATGGA C-3′, and downstream primer: 5′-TTTGG ATTCA TAGG C GAGAA TCCC ACAT-3′.

Total RNA was extracted from waxy maize inbred line "W1" using the plant RNA extract kit, reverse transcription was performed to obtain cDNA, and PCR amplification was performed using the cDNA template. The reaction system, totally 50 µL, included ddH_2O (37.6 µL), 10×buffer (Mg^{2+} plus, 5.0 µL), dNTP mixture (2.5 mmol/L, 4.0 µL), upper primer (50 pmol/L, 1.0 µL), lower primer (50 pmol/L, 1.0 µL), cDNA template (50 ng/µl, 1.0 µL), and EXTaq enzyme (5 U/µL, 0.4 µL). The reaction program was 94°C for 5 min; 35 cycles (94°C for 30 s, 56°C for 30 s, and 73°C for 90 s); and 72°C for 10 min for final extension.

PCR products were separated by 1% agarose electrophoresis, recycled, and ligated to pMD18-T vector, forming pMD18-T-SBE2a, and the construct was transferred to *E. coli* DH5α. Positive white colonies were selected and cultured, plasmid DNA was extracted using the alkali lysis method, and DNA was identified via restriction enzymes and gene sequencing (TaKaRa Company Dalian, Chima). Nucleic acid sequences were analyzed using DNASIS (Medprobe, UK) software.

RNAi vector constructs

The RNAi construct pRSBE2a, consisting of the corn *sbe2a* gene, is shown in Figures 1a and b. The pMD18-T-SBE2a construct containing the *sbe2a* gene was digested with *Xba*I and *BamH*I. The modified pCAMBIA1301 vector, in which *hygromycin* resistance gene was removed, was also opened by the same endonucleases. The retrieved fragments containing the *sbe2a* fragments were forward and inversely inserted into the downstream adjacent to *35S* promoter in modified pCAMBIA1301 vector forming pRSBE2a. The construct was transferred to DH5α, screened on Luria Bertani (LB) medium supplemented with kanamycin (50 µg/mL). Five positive colonies were screened out. To verify the constructs, plasmid DNA of positive colonies was extracted and digested. Plasmid was extracted using the mini DNA extraction kit, PCR amplification was performed using P1, P2 primers and pRSBE2a as templates. PCR products were digested with *Xba* I+*BamH* I and *Sac* I+*Xho* I, respectively, to identify the sense and antisense fragments.

Pollen tube pathway transformation of maize by RNAi vector

An alkaline lysis method was used to extract plasmid DNA in a large scale, and then DNA introductory solution was prepared to a concentration of 500 to 1,000 µg/mL. In 8 to 12 h after self-pollination of recipient maize inbred line Tie7922, the stylus was removed, then 500 µL of DNA introductory solution was dropwise added to the incision, packaging quickly. One hour later, DNA solution was dropwise added again. Equivalent saline-sodium citrate (SSC) solution without DNA was served as the control which was treated with the same procedures.

Detection of transgenic T1 plants

Clusters with antibiotic resistance and good seeds were selected from Tie7922 T0 plants, which experienced pollen tube pathway-mediated transformation in the farm field, and then were planted in greenhouse. As transgenic T1 plants came into five to six leaves, every three plants were divided into one group. Genomic DNA was extracted from the mixed leaves of regenerative plants using a modified cetyltrimethylammonium bromide (CTAB) method. Primers were designed for the *35S* promoter sequence of pRSBE2a. The primer sequences were as follows: P3 upstream, 5'-GTGAAT-CCGCACCTCT-3' and P3 downstream, 5'-ATCGCCGCTTT-GGACATA-3'. PCR amplification was performed for primary screening of transgenic lines. Expression vector pCAMBIA1301 and non-transgenic plant genomic DNA served as the negative and

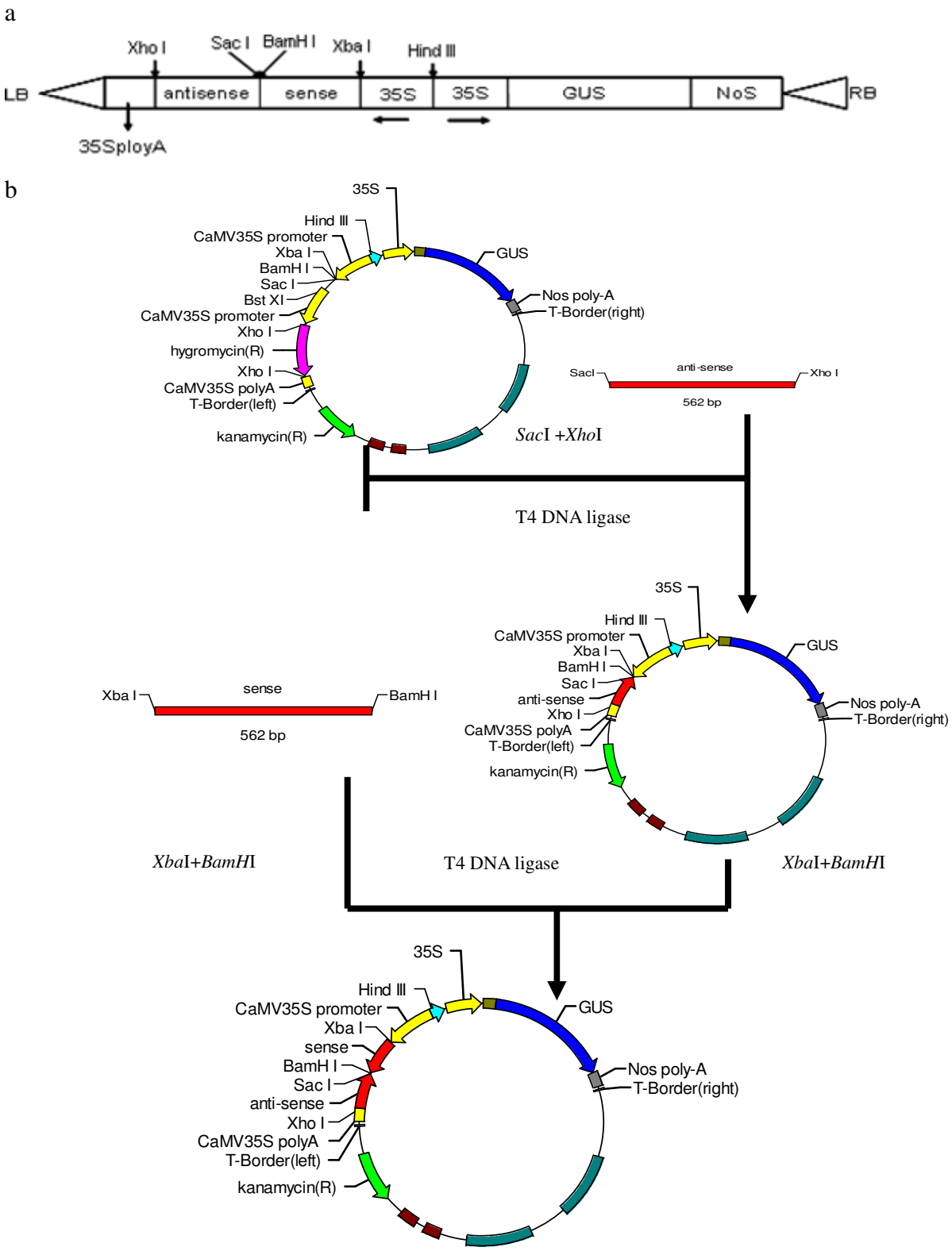

Figure 1. (a) The structure map of the T-DNA region of the RNAi vector pRSBE2a, (b) diagram of constructing corn starch branch enzyme gene RNAi expression vector (pRSBE2a).

blank controls, respectively. The reaction system, totally 25 µL, included ddH_2O (15.25 µL), 10×buffer (2.5 µL), $MgCl_2$ solution (2

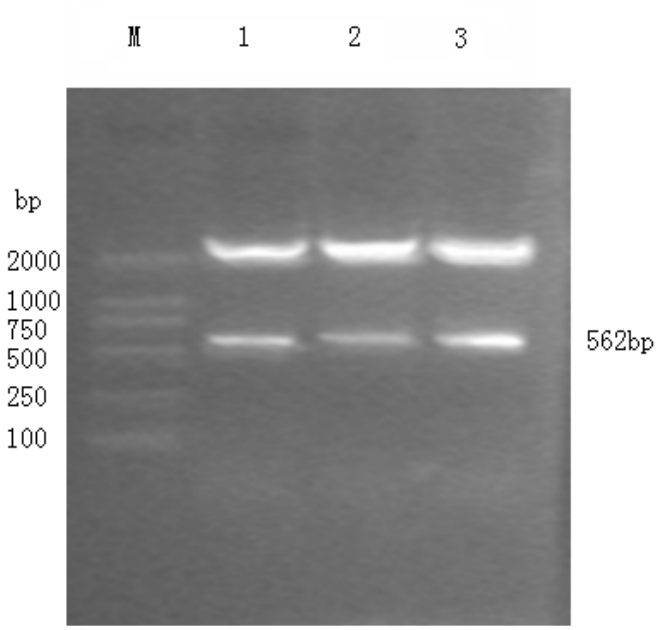

Figure 2. Restriction enzyme analysis of the reconstructs. M, DNA Marker DL-2,000; 1 to 3, pMD18-T-SBE2a/*Apa* I+ *Xba*

μL), dNTP mixture (2.5 mmol/L, 2.0 μL), upper primer (25 pmol/L, 1.0 μL), lower primer (25 pmol/L, 1.0 μL), DNA template (25 ng/μl; 1.0 μL), and EXTaq (5 U/μL; 0.25 μL). The reaction program was 94°C for 10 min; 35 cycles (94°C for 1 min, 56°C for 40 s, and 72°C for 3 min); and 72°C for 10 min for final extension.

A random single plant of each positive group was detected by Southern blotting. The 836 bp promoter fragment on the pRSBE2a was amplified by PCR and labeled with DIG to be used as the probe for the Southern blotting. Genomic DNA was extracted from PCR-positive plants, digested with *BamH* I, separated on 0.8% agarose, then transferred to nylon membranes in 20xSSC solutions, fixed at 80°C for 2 h, and hybridized with *35S* promoter probes (830 bp). The DIG labeling and testing kit was used for probe labeling, hybridization, and color development as manufacture's instructions.

Reverse transcript PCR analysis of transgenic T1 kernels *sbe2a* mRNA accumulation in transgenic plants were detected by PCR method, using a non-transgenic plant as the blank control and corn *EF-1a* gene (213bp) as the inner control. Using the Plant RNA Extract Kit, total RNA was extracted from the kernels of transgenic T1 plants 20 days after pollination. cDNA was synthesized. PCR amplification of *sbe2a* fragment was conducted using cDNA as template. The *sbe2a* primer sequences were as follows: 5'-CGTGT-AAAGATACGGATGGAC-3' (upstream) and 5'-ATAGGCGAGAAT-CCCACAT-3' (downstream), with an expected product of 562bp. The *EF-1*a primer sequences were as follows: 5'-GCTTCACGT-CCCAGGTCATC-3' (upstream) and 5'-TAGGCTTGGTGGGT-ATCATC-3' (downstream), with an expected product of 213 bp (Kirchberger et al., 2007). The reaction systems were same as immediately abovementioned. The reaction program was 94°C for 5 min, 28 cycles (94°C for 30 s, 54°C for 30 s, 72°C for 1 min), and 72°C for 10 min for a final extension.

Analysis of SBE activity in transgenic T1 kernels

Five kernels of transgenic maize 20, 25, and 30 days after pollination (15 kernels totally), were harvested, weighed, and soaked in 0.05 mol/L citric acid buffer solution (pH 7.0), ground and homogenized in an ice bath (22 mL totally). Homogenates were then precipitated by centrifugation at 20,000× *g* for 20 min. Supernatant contained the gross enzyme extract. SBE activity assay was performed according to Sestili et al. (2010) report. The SBE activity of non-transgenic plant served as control. The percent of SBE activity decrease was calculated as follows: ΔOD_{660} % = (control OD_{660} – experimental OD_{660})/control OD_{660} × 100%.

Starch content of transgenic T1 kernels

The amylose and amylopectin contents were determined using a dual wavelength method. For amylose content determination, the major and contrast wavelengths were 620 and 480 nm, respectively; for amylopectin, the major and contrast wavelength were 550 and 760 nm respectively. Each treatment was repeated three times. Total starch was the sum of amylopectin and amylose.

Analysis of transgenic T2 plants and kernels

Transgenic T1 plant corncob was covered with a plastic bag to allow inbreeding. A total of 20 random seeds of transgenic T1 plants (Tie7922-1, Tie7922-2, Tie7922-3, and Tie7922-4) were planted and grew up into transgenic T2 plants, respectively. Genomic DNA was extracted from the leaves of the transgenic T2 plants for PCR detection. SBE activity and starch content in the transgenic T2 kernels of PCR-positive T2 plants were detected as done to transgenic T1 kernels.

Statistical methods

SPSS10.0 software was used for statistical analysis. Chi-square fitness test was performed to analyze the fitness of transgenic T2 plants to the Mendel law. Student t-test was used for comparing SBE activity and starch contents between transgenic kernels and non-transgenic kernels, respectively. $P < 0.01$ indicated statistically significant difference.

RESULTS

Cloning and sequencing of sbe2a fragment

As shown in Figure 2, specific and pure fragments of approximately 562 bp were obtained, as expected. After recycling, ligation into the vector, and transfer into *E. coli*, five positive colonies were obtained by screening. An insert fragment of approximately 562 bp was obtained via restriction digestion, suggesting that the target fragment had been inserted into the vector (Figure 2).
The sequencing results were as follows:

ATAGGCGAGA ATCCCACATC CAATGATGGC
CTCGTGGACC ACCATGGAAG
TAATGTGTACGGTGCCATC GAAACCATTC
AAACCATCCA AGGTATTATT TGATGAATGA
CTATGAACAA TATCCATAAG CACTAGCAAG
CCAAGCTCAT GCGCTTTATC AATAAGAGATT
TTAGGTCCT CTGGAGTCCC AAAACGGCTA
CTTGGGGCAA AAAAATTCGT AACATGGTA

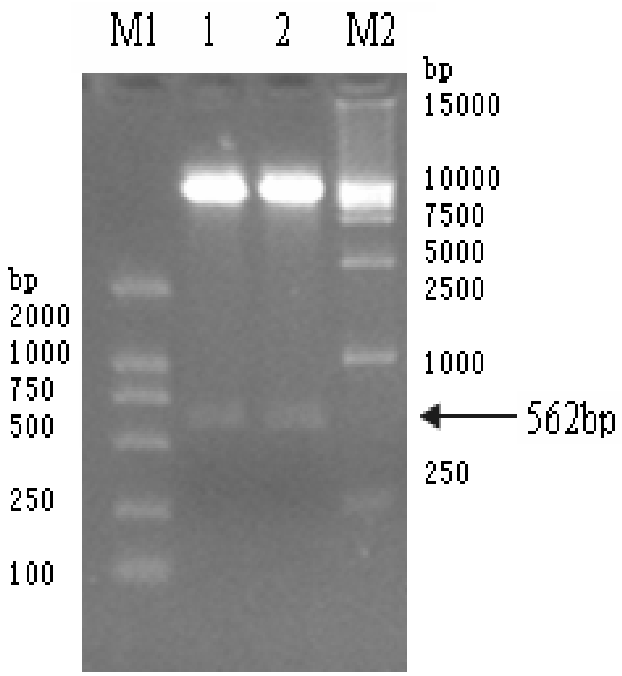

Figure 3. Restriction enzyme analysis of the reconstructs. M, DNA Marker DL-2,000, DL-15,000; 1, pRSBE2a/*Sac*I+*Xho*I; 2, pRSBE2a/*Xba* I+*BamH* I.

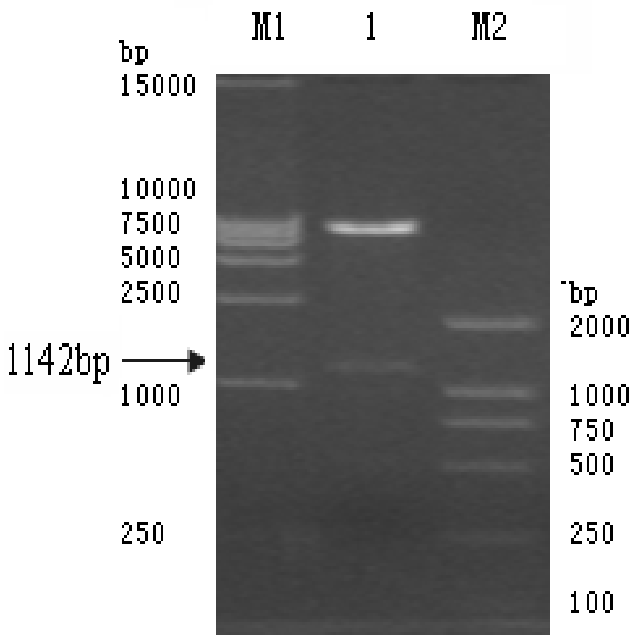

Figure 4. Restriction enzyme analysis of the reconstructs. M1 and M2, DNA Marker DL-2,000, DL-15,000; 1: pRSBE2a/*Xba* I + *Xho* I.

CCCAAAGCTTG CATAATAAGA GTGTTCCTGG
ATTGCCATTA TCTGTACTGC ATTGTATCCAAG
CTTTTTAATTCTTGGAAG CACCTCATCT
CTGAAGTTAG CATATGTATT TATCTTTG
GTTCCGGGCTAC TCATTCCAAC ATGTGATTCA
TATATCCGCA GTGACTTGGG CCGCTTAGGT
TGAGGGTGTT TGAATACATA TTTCTCCTCT
TCAGGTGGGT CATAATATAT ACCGTTGTAT
GGTATTTCAC CTGGAGCCTG CACAGAAAAC
TTGATCCAGG CAGGAATGGAA TCCTTAACA
CCAGATGGTGTGTCCATCCG TATCTTTACA (562 bp).

Sequencing analysis showed that the interest fragment in the present paper had only one different base when compared to that reported in NCBI GenBank (U65948), that is, the homology was up to 99.8%. This implies that the cloned sequence was the same as the corn *sbe2a* fragment.

Construcion of pRSBE2a

Digestion produced approximate 562 bp fragments for all positive clones (Figure 3). A specific 1,110 bp fragment was produced after digestion with *Xba* I+*Xho* I to identify the interference-potent fragments (Figure 4). These results demonstrate that the interest fragments have been successfully inserted into the downstream site of *35S* promoter, which was carried on plant expression vector pCAMBIA1301, namely, *sbe2a*-containing RNAi expression vector pRSBE2a.

PCR detection of transgenic T1 plants

A total of 800 seeds of T0 plants were selected and planted in the farm to grow transgenic T1 plants. The results verified total four resistant transgenic T1 plants (Tie7922-1, Tie7922-2, Tie7922-3 and Tie7922-4) and primarily demonstrated that the exogenous genes had been integrated into maize genomes.

Southern blotting

DNA was extracted from leaves of each positive transgenic T1 plant, and digested with *BamH* I. As shown in Figure 5, hybrid signals appeared for each positive plant, in accordance with the results of PCR detection, whereas the signal was not present for non-transgenic plants. This result verifies that the exogenous gene was integrated into the genome of the transgenic plant and it implies that the exogenous gene may integrate one or two copies into the transgenic maize genome.

RT-PCR analyses of transgenic T1 kernels

The results of RT-PCR on transgenic T1 maize kernels

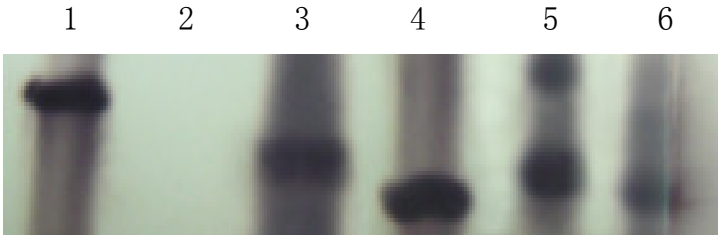

Figure 5. Southern blot of transgenic T1 plants. 1, Positive control; 2, non-transgenic plant; 3 to 6, transgenic plants (Tie7922-1, Tie7922-2, Tie7922-3 and Tie7922-4).

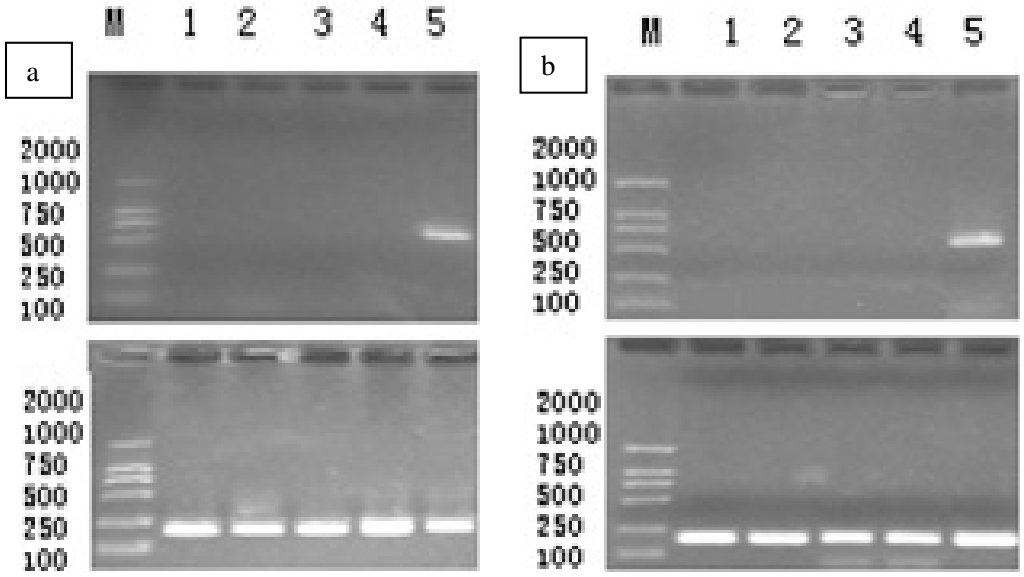

Figure 6. Reverse Transcript PCR analysis of transgenic T1 kernels. (a) 28 cycle reaction, (b) 30 cycle reaction. M, DNA marker DL-2,000; 1 to 4, transgenic kernels (Tie7922-1, Tie7922-2, Tie7922-3, Tie7922-4), 5, non-transgenic kernel; *Sbe2a* fragment, 562bp; *EF-1*a, 213bp.

are shown in Figure 6. The endogenous SBE mRNA content was decreased significantly in the transgenic plants, indicating the exogenous gene was transcripted normally to cause the endogenous *sbe* mRNA degradation.

SBE activity and starch content in transgenic T1 kernels

Kernels were harvested from transgenic plants (Tie7922-1, Tie7922-2, Tie7922-3, and Tie7922-4). The SBE activities for transgenic T1 kernels were 0.03556U, 0.04025U, 0.02231U and 0.0202U (n = 3), respectively, and 0.12721U for control (Figure 7a). SBE activities were reduced by 60.4, 55.1, 75.1 and 77.5% (average 65.3%), respectively. SBE activities in transgenic T1 kernels were significantly lower than that of the non-transgenic plant ($P < 0.01$ for each); suggesting that the translation of endogenous SBE mRNA was inhibited to some extent by the RNAi vector and SBE activity was also reduced.

The total starch contents of transgenic Tie7922-1, Tie7922-2, Tie7922-3, and Tie7922-4 were 670, 680, 690, and 680 mg/g DW (kernels), respectively, similar to that of control; however, the percent contents of amylose were 38.9, 35.5, 48.8, and 50.9%, respectively, higher than 27.1% for the non-transgenic plant ($P < 0.01$ for each; Figure 7b); the at most increase was 87.8% (50.9 vs. 27.1%) and the average growth was 60.6% (43.6 vs. 27.1%).

PCR analysis of transgenic T2 plant

PCR results for transgenic T2 plants are shown in Table 1. The results demonstrate that the exogenous gene was transmitted on to transgenic T2 plants, and the segregation ratio was complied with Mendel law (Chi-square = 6.37, $P < 0.01$).

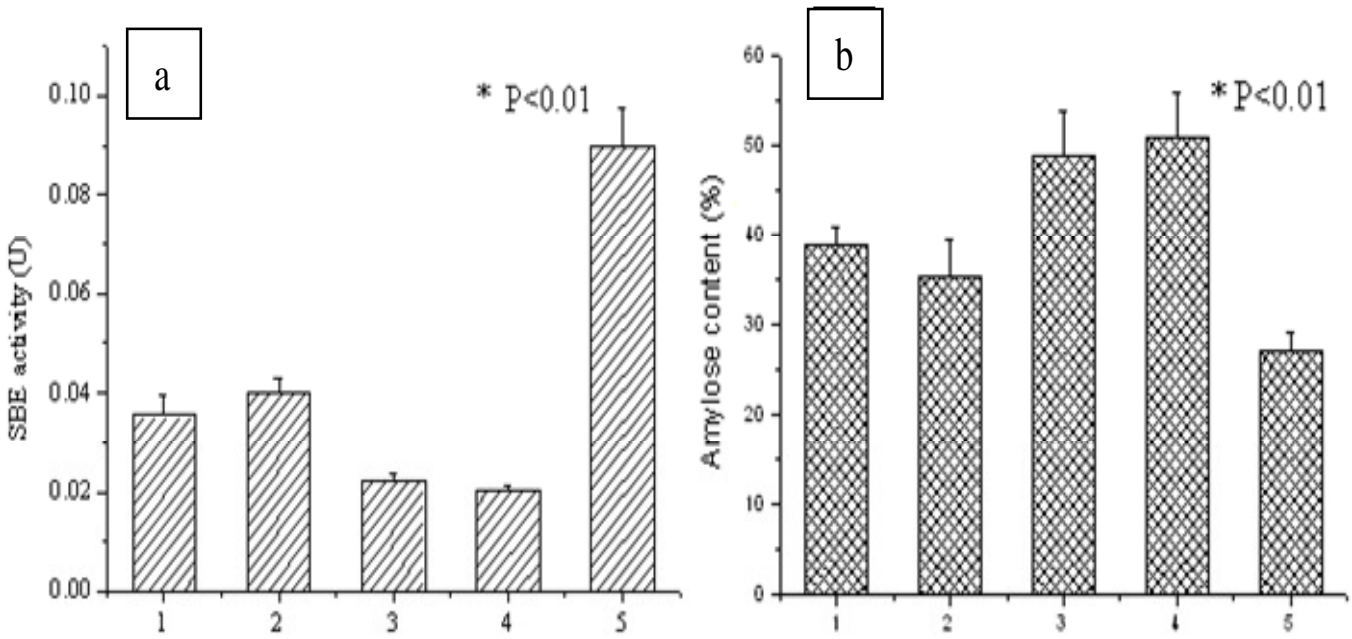

Figure 7. SBE activity and amylose contents in transgenic T1 kernels. (a) SBE activity; (b) Amylose contents. 1 to 4, Transgenic kernels (Tie7922-1, Tie7922-2, Tie7922-3, Tie7922-4); 5, non-transgenic kernel.

Table 1. PCR analysis of transgenic T2 plants.

T_2 line	No of seed	No. of seedling	No. of positive plant	No. of negative plant	Positive/negative
Line 1	20	20	15	5	3:1
Line 2	20	19	14	5	2.8:1
Line 3	20	17	13	4	3.2:1
Line 4	20	18	14	4	3.5:1

SBE activity and starch content in transgenic T2 kernels

In transgenic T2 plants, Tie7922-1-1, Tie7922-2-1, Tie7922-3-1, and Tie7922-4-1 plants were detected to have the down-regulated endogenous *sbe* mRNA, so their kernels were selected for the analyses of SBE activity and starch content.

The SBE activities for transgenic T2 kernels were 0.03855U, 0.04106U, 0.02354U, and 0.02015U (n = 3), respectively, and 0.09152U for the non-transgenic plant (Figure 8a); the SBE activities were reduced by 57.8, 55.1, 74.3, and 77.9%, respectively, when compared to that of the non-transgenic plant ($P < 0.01$ for each).

The total starch contents of transgenic T2 kernels were 680, 680, 670, and 690 mg/g DW (kernels), respectively, similar to that of blank control; however, the percent contents of amylose were 36.9, 34.8, 48.5, and 51.5%, respectively, significantly higher than 27.5% for the non-transgenic plant ($P < 0.01$ for each; Figure 8b); the maximum increase was 87.2% (51.5 vs. 27.5%) and the average increase was 56.1% (42.9 vs. 27.5%).

DISCUSSION

In the present paper, *sbe2a* gene was cloned and the corresponding RNAi vector was constructed. Using pollen tube pathway transformation, the RNAi vector was successfully imported into maize inbred lines.

Stoutjesdijk et al. (2002) achieved the suppression of *FAD2* gene in Arabidopsis by using RNAi technology, and this depression effect could be transmitted to the offspring. This finding reveals the potential of RNAi technology to apply in crop seed quality and properties improvement for the first time. Andika et al. (2005) used RT-PCR to clone rape *PEP* gene fragment and construct RNAi vector of *PEPase* gene to suppress *PEPase* gene expression in rape, making the metabolic flux deflect towards oil synthesis, consequently increasing oil content in rapeseed. Generally, RNAi as a focus issue in molecular biology and genetic engineering opens up a new approach in fundamental and applied research.

The results in the present paper have shown that SBE activity was significantly decreased (by an averaged 65.3 and 65.3%, respectively) in transgenic T1 and T2 kernels

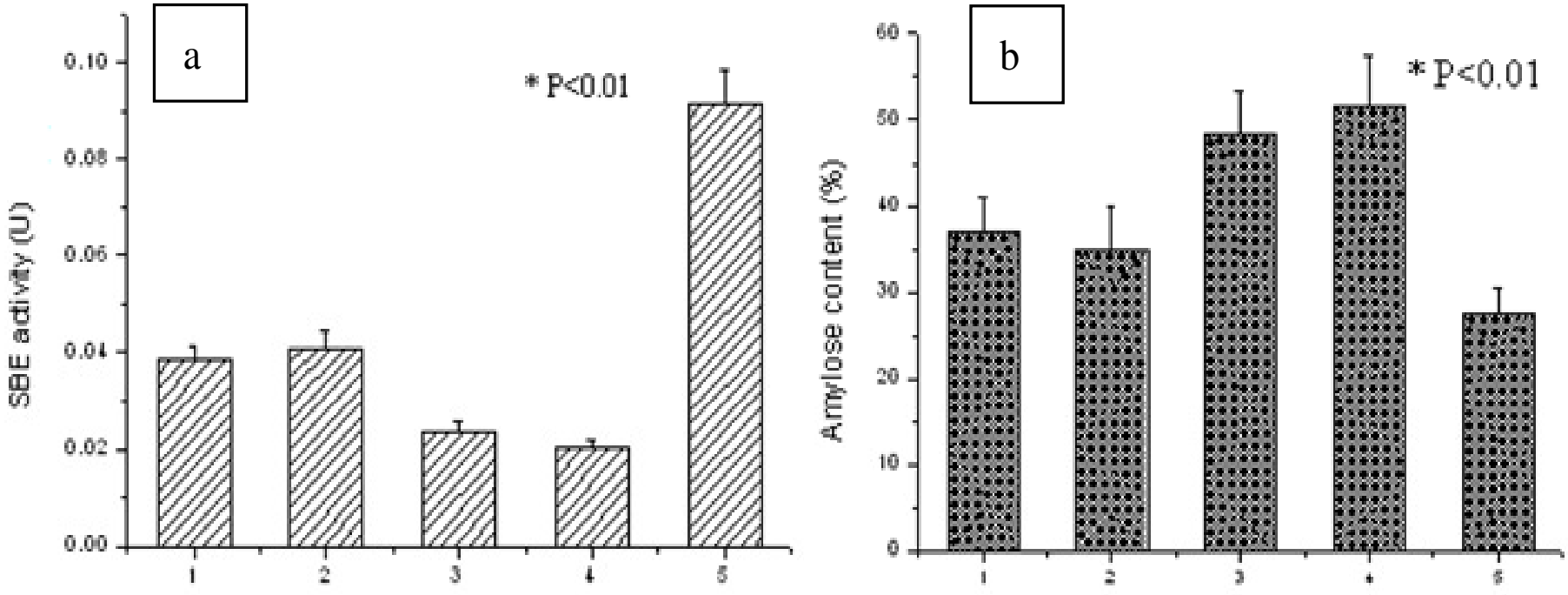

Figure 8. SBE activity and amylose contents in transgenic T2 kernels. (a) SBE activity; (b) amylose contents. 1 to 4, Transgenic kernels (Tie7922-1-1, Tie7922-2-1, Tie7922-3-1, Tie7922-4-1); 5, non-transgenic kernel.

when compared to the non-transgenic plant, suggesting that the translation of endogenous *Sbe2a* mRNA was inhibited by RNAi expression vector effectively. Meanwhile, the content of amylose was significantly increased (by an averaged 60.6 and 56.1%; 87.8% at most) in transgenic T1 and T2 kernels under the condition of the total starch content. These findings indicate that RNAi technology can effectively regulate the corn starch synthesis. It inhibits the synthesis of amylopectin and greatly enhances the amylose content in order to produce high-amylose maize inbred lines and provide a new way to improve corn high amylose. Breeding of high-amylose maize hybrid is in progress for the further studies.

Conclusion

In the present study, a portion of *sbe2a* gene fragments were forward and inversely inserted into plant expression vector and an RNAi vector for *sbe2a* was successfully constructed. As these reconstructs were successfully introduced into maize inbred lines by pollen tube pathway transformation, gene expression was suppressed by dsRNA that was formed by sense and anti-sense RNA annealing; thereby leading to the specific posttranscriptional degradation of homologous mRNA and leading to efficient and specific *sbe2a* gene repression. RNAi as an efficient means for gene silencing can regulate the metabolic pathway of corn starch and inhibit target gene expression. By inhibiting *sbe2a* gene expression, the RNAi vector can effectively reduce SBE activity to improve the corn amylose content.

ACKNOWLEDGEMENTS

This study is funded by National Transgenic

Special Project (No. 20082x08003-005); Jilin Provincial Division of Finance Project (No. 200806); Provincial Division of Science and Technology Project (No. 20095044).

REFERENCES

Andika IB, Kondo H, Tamada T(2005). Evidence that RNA silencing-mediated resistance to Beet necrotic yellow vein virus is less effective in roots than in leaves. Mol. Plant Microbe In. 18: 194-204.

Blauth SL, Yao Y, Klucinec JD, Shannon JC, Thompson DB, Guilitinan MJ (2001). Identification of Mutator Insertional Mutants of Starch-Branching Enzyme 2a in Corn. Plant Physiol. 125: 1396-1405

Casey J, Slattery I, Kavakli H, Okita TW (2000). Engineering starch for increased quantity and quality. Trends Plant Sci. 5: 291-298.

Denver K, Johnson P, Samuel Z, Alison MS (2001). The control of amylose synthesis. Plant Physiol. 158: 479-487.

Yandeau-Nelson M, Laurens L, Shi Z, Xia H, Smith AM, Guiltinan M (2011). Starch Branching Enzyme IIa is required for proper diurnal cycling of starch in leaves of *Zea mays*. Plant Physiol. 156: 479-490

Kirchberger S, Leroch M, Huynen MA, Wahl M, Neuhaus HE, Tjaden J (2007). Molecular and biochemical analysis of the plastidic ADP-glucose transporter (ZmBT1) from Zea mays. J. Biol. Chem. 282: 22481-22491

Koga A, Ishibashi T, Kimura S, Uchiyama Y, Sakaguchi K (2006). Characterization of T-DNA insertion mutants and RNAi silenced plants of Arabidopsis thaliana UV-damaged DNA binding protein 2 (AtUV-DDB2). Plant Mol Biol. 61(1-2): 227-240.

Nunes AC, Vianna GR, Cuneo F, Amaya-Farfn J, deCapadeville G, Rech EL, Aragáo FJ (2006). RNAi-mediated silencing of the myo-inosit-1-phosphate synthase gene (GmMIPS1) in transgenic soybean inhibited seed development and reducedphytate content. Planta, 224: 125-132.

Qiao F, Yang Q, Wang CL, Fan YL, Wu XF, Zhao KJ (2007). Modification of plant height via RNAi suppression of OsGA20ox2 gene in rice. Euphytica, 158(1-2): 35-45

Schweizer P, Pokorny J, Schulze-Lefert P, Dudler R (2000). Double-stranded RNA interferes with gene function at the single-cell revel in cereals. Plant J. 24: 895-903

Sestili F, Janni M, Doherty A, Botticella E, D'Ovidio R, Masci S, Jones HD, Lafiandra D (2010). Increasing the amylose content of durum wheat through silencing of the SBEIIa genes. BMC Plant Biol. 10: p. 144.

Smith AM, Denver K, Martin C (1997). The synthesis of the starch granule. An. Rev. Plant Physiol. Plant Mol. Biol. 48: 67-87

Stoutjesdijk PA, Singh SP, Liu Q, Hurlstone CJ, Waterhouse PA, Green AG (2002). hpRNA-mediated targeting of the Arabidopsis FAD2 gene gives highly efficient and stable silencing. Plant Physiol. 129: 1723-1731

Sunikumar G, Campbell LM, Puckhaber L, Stipanovic RD, Rathore KS(2006). Engineering cotton seed for use in human nutrition by tissue-specific reduction of toxic gossypol. Proc. Nat. Acad. Sci. USA. 103: 18054-18059.

Visser RGF, Jacobsen E (1993). Towards modifying plants for altered starch content and composition. Trends Biotechnol. 11: 63-68