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Development of Marker-free *TaGlu-Ax1* Transgenic Rice Harboring a Wheat High-molecular-weight Glutenin Subunit (HMW-GS) Protein

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High-molecular-weight glutenin subunits (HMW-GSs) are extremely important determinants of the functional properties of wheat dough. Transgenic rice plants containing a wheat *TaGlu-Ax1* gene encoding a HMG-GS were produced from the Korean wheat cultivar 'Jokyeong' and used to enhance the bread-making quality of rice dough using the *Agrobacterium*-mediated co-transformation method. Two expression cassettes with separate DNA fragments containing only *TaGlu-Ax1* and hygromycin phosphotransferase II (*HPTII*) resistance genes were introduced separately into the *Agrobacterium tumefaciens* EHA105 strain for co-infection. Rice calli were infected with each EHA105 strain harboring *TaGlu-Ax1* or *HPTII* at a 3:1 ratio of *TaGlu-Ax1* and *HPTII*. Among 210 hygromycin-resistant T_0 plants, 20 transgenic lines harboring both the *TaGlu-Ax1* and *HPTII* genes in the rice genome were obtained. The integration of the *TaGlu-Ax1* gene into the rice genome was reconfirmed by Southern blot analysis. The transcripts and proteins of the wheat *TaGlu-Ax1* were stably expressed in rice T_1 seeds. Finally, the marker-free plants harboring only the *TaGlu-Ax1* gene were successfully screened in the T_1 generation. There were no morphological differences between the wild-type and marker-free transgenic plants. The quality of only one HMW-GS (*TaGlu-Ax1*) was unsuitable for bread making using transgenic rice dough. Greater numbers and combinations of HMW and LMW-GSs and gliadins of wheat are required to further improve the processing qualities of rice dough. *TaGlu-Ax1* marker-free transgenic plants could provide good materials to make transgenic rice with improved bread-making qualities.

Key words : Co-transformation, high-molecular-weight glutenin subunit (HMW-GS) protein, marker-free transgenic rice, wheat

Introduction

Rice is the most important cereal in the developing world and is a staple food consumed by a large part of world human population [10]. Rice flour is one of the most valuable cereal flours from a nutritional viewpoint due to its hypoallergenic proteins and low calcium content, moreover the absence of gluten [12]. Rice flour is used in many food products and improving rice quality is of great relevance to many Asian countries. However, dough made from rice lacks extensibility and elasticity, whereas that of wheat is suitable for many food products including breads and noodles. Wheat flour is different from other cereal flours, including rice, because it contains gluten that gives it the elasticity and extensity required for bread-making [5].

Gluten consists mainly of two types of seed storage proteins, the glutenins and the gliadins. Glutenins are classified into high-molecular-weight glutenin subunits (HMW-GS) and low-molecular-weight glutenin subunits (LMW-GS). Although the HMW-GS contribute only about 5% of the total protein in mature wheat kernels [28], the elasticity of wheat dough depends mainly on the HMW-GS, so their networking is important determinants of bread-making quality [23].

The HMW-GS are encoded by the Glu-A1, Glu-B1 and Glu-D1 genes on the long arm of chromosomes 1A, 1B and 1D, respectively [13]. Each locus includes two genes linked

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together encoding two different types of HMW-GS, x- and y-type subunits [24]. Several HMW-GS genes have been shown to be functional when transformed into *Escherichia coli* [11], tobacco [25], wheat [2, 3, 5, 6] and tritordeum [26].

Development of transgenic plants increasing bread-making quality using various HMW-GS genes is good way to solve lacks of rice extensibility and elasticity. Araki's [4] group has developed transgenic rice using transformation-competent artificial chromosome (TAC) clones harboring a HMW-GS and LMW-GS genes from wheat genomic DNA. All transgenic lines had HMW-GS subunit proteins in the rice endosperm, and the expressed proteins are processed at the same site as the mature protein in wheat seeds.

The persistence of selectable marker genes in transgenic crops destined for field cultivation and human food leads to serious public concerns about the safety of transgenic crops, even though several risk-assessment reports [17, 27] have shown that neither the genes nor their products are harmful to human or environmental health. Moreover, generating marker-free transgenic plants responds not only to public concerns over the safety of genetically engineered crops, but supports multiple transformation cycles for transgene pyramiding. Repeated use of the same promoter and a polyadenylation signal for different selectable marker genes could result in transcriptional gene silencing [15]. Therefore, eliminating selectable marker genes is crucial for stacking multiple traits in a transgenic plant.

As one of major Gluten proteins, the HMW-GS Ax1 has been reported that there is associated with processing properties. Although co-expression of *TaGlu-Ax1* and PINA in durum wheat have combined effects on dough mixing behaviors with a better dough strength and resistance to extension than those from lines expressing *TaGlu-Ax1* or *Pina* [19], the expression of *TaGlu-Ax1* is no doubt to influence the processing properties. Overexpression of HMW-GS Ax1 in several durum wheat cultivars resulted in increased dough strength [13].

In this study, we generated marker-free transgenic rice expressing the wheat HMW-GS protein without any herbicide or antibiotic resistance marker genes using the co-transformation method. The marker-free transgenic plant expressing *TaGlu-Ax1* gene is critical material for generating transgenic plant advanced quality processing of bread and noodle without antibiotic markers. Moreover, the marker-free transgenic rice developed in this study should provide useful for improving processing quality in rice breeding program.

Materials and Methods

Cloning of the wheat *TaGlu-Ax1* glutenin gene

'Jokyeong' (*Triticum aestivum* L. cv. Jokyeong) was used for cloning the *TaGlu-Ax1* gluenin gene. The *TaGlu-Ax1* gene was amplified by polymerase chain reaction (PCR) of genomic DNA using the primers *TaGlu-Ax1-CF (primer sequences:* 5'-TCATCACCCACAACACCGAGCA-3') and *TaGlu-Ax1-CR* (primer sequences: 5'- AGCTGCAGAGAGTTCTAT CACTG-3'), which were designed from a sequence on GenBank (accession no. X61009). The PCR temperature cycling conditions were 4 min at 94°C, followed by 35 cycles of 94°C for 30 sec, 60°C for 30 sec, 72°C for 2 min, and a final extension at 72°C for 10 min. The amplification products were separated on a 1% agarose gel and visualized with EtBr. The amplified products were sub-cloned using a TOPO TA Cloning kit for sequencing (Invitrogen, Carlsbad, CA, USA).

DNA constructs

To make a marker-free vector, we first inserted *TaGlu-Bx7*-own promoter from wheat cultivar 'Jokyeong' into the *pBTEX* binary vector, which modified from *pCAMBIA1300* binary vector. The *HPTII* expression cassette (*CaMV 35S* promoter-*HPTII* gene-*CaMV 35S* terminator) in the *pBTEX* binary vector was removed by *Xho*I and *EcoR*I restriction enzyme treatment. After klenow enzyme treatment for blunt ligation, the vector was self-ligated. Then, amplified the *TaGlu-Ax1* gene with the *EcoR*I and *Kpn*I restriction enzyme sites was constructed into *pBTEX* binary vectors under the control of *TaGlu-Bx7*-own promoter (Fig. 1A). The positive selectable marker cassette for co-transformation was used by an empty *pBTEX* binary vector (Fig. 1B).

Agrobacterium handling

Competent *Agrobacterium tumefaciens* EHA105 was trans-

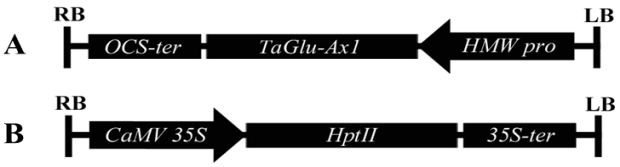

Fig. 1. Vector constructs expressing the *TaGlu-Ax1* (upper panel) and hygromycin phosphotransferase II (*HPTII*) (lower panel) genes in the binary vectors. HMW pro, *TaGlu-Bx7*-own promoter; OCS-ter, octopine synthase terminator; CaMV 35S, cauliflower mosaic virus promoter; 35S-ter, 35S terminator; RB, right border; LB, left border.

formed with *TaGlu-Ax1*- cloned binary vector and an empty vector containing *HPTII* for the selectable marker using the freeze-thaw method [7]. T_0 plants were selected on YEP media containing kanamycin (50 mg/l). Transformation was confirmed by PCR amplification of plasmids mini-prepped from each *Agrobacterium* strain [3].

Rice co-transformation

Mature seeds of *Oryza sativa* L. subsp. *japonica* var. Dongjin were used to induce callus formation on callus induction (CI) medium [N_6 salts [9] with vitamins, 2.5 g/l proline, 2 mg/l 2,4-D, 0.5 g/l casamino acid, 30 g/l sucrose and 2 g/l gelrite, pH 5.7]. After 21 days of incubation in the dark at 25°C, the scutellum-derived calli were excised and preincubated on CI medium for 1 week. Agrobacterial cells were grown on YEP solid medium containing antibiotics at 25°C for 2 days. And then, agrobacterial cells were resuspended in suspension medium (N_6 salts with vitamins, 2 mg/l 2,4-D, 0.5 g/l casamino acid, 30 g/l sucrose, and 10 g/l glucose, pH 5.7) with 200 μM acetosyringone as a final concentration. After two *Agrobacterium* cells were mixed in a 3:1 ratio of EHA105 with *TaGlu-Ax1* gene expressing cassette and EHA105 with *HPTII* gene expressing cassette, the calli were transformed by swirling in the mixture of *Agrobacterium* cultures for 30 min. The calli were blotted on Whatman no. 1 paper and cocultivated on the cocultivation medium (N_6 salts with vitamins, 2 mg/l 2,4-D, 0.5 g/l casamino acid, 30 g/l sucrose, 10 g/l glucose, and 2 g/l gelrite, pH 5.2 with 200 μM acetosyringone as a final concentration). After 3 days, the calli were washed with liquid CI medium supplemented with 250 mg/l cefotaxime and 150 mg/l and placed on the selection medium (CI medium supplemented with 50 mg/l hygromycin, 250 mg/l cefotaxime). After selection and regeneration, the regenerated plantlets were acclimatized and grown in a greenhouse.

PCR analysis of T_0 plants

PCR was performed with the GeneAmp System 9700 (Applied Biosystems, Foster City, CA, USA) with a gene-specific primer set (*TaGlu-Ax1*; forward 5′- TCATCACCCACAA CACCGAGCA -3′, reverse 5′- GACCTTGTCCTGGTTGCT GTCTTTG -3′, *HPTII*; forward 5′-CGCTTCTGCGGGCGA TTT-3′, reverse 5′-CCCATTCGGACCGCAAGGA -3′) and EF Taq DNA polymerase (Solgent Co. Seoul, South Korea). Each reaction mixture (30 μM) consisted of 10 mM Tris-HCl (pH 9.0), 1.5 mM $MgCl_2$, 40 mM KCl, 250 μM dNTPs, and 1 U Taq DNA polymerase. Amplified products were separated on a 1% agarose gel, stained with EtBr, and visualized with a UV illuminator.

Southern hybridization analysis

Rice genomic DNA was prepared by the CTAB extraction method [31]. Aliquots of 5 μg of purified DNA were digested with restriction endonuclease (*EcoRI*), size-fractionated on a 0.8% agarose gel, and the DNA was transferred to a nylon membrane through capillary blotting in 10× SSC (Gene Screen, DuPont, Wilmington, DE, USA). The blots were labeled using AlkPhos Direct (Amersham, GE Healthcare. Piscataway, NJ, USA) according to the manufacturer's instructions. After hybridization, the filters were washed for 30 min at 55°C to remove unlabelled probe. Subsequently, CD-star Detection Reagent (Amersham, GE Healthcare. Piscataway, NJ, USA) was used to detect and generate signals.

RNA extraction and RT-PCR analysis

T_1 generation seeds were frozen in liquid nitrogen and then ground to powder using a mortar and pestle. Total RNA was extracted using a method reported previously [34]. The isolated RNA preparations were then reverse-transcribed with oligo-dT primer and a First Strand cDNA Synthesis kit for RT-PCR (Roche Co., Basel, Switzerland) with gene-specific primers. The primers were as follows: *TaGlu-Ax1* forward 5′- TCATCACCCACAACACCGAGCA -3′, *TaGlu-Ax1* reverse 5′- GACCTTGTCCTGGTTGCTGTCT TTG -3′; *OsActin* primers were used as internal standards for mRNA expression profiling [21, 33]. The PCR conditions consisted of initial denaturation at 94°C for 4 min, followed by 30 cycles at 94°C for 1 min, 60°C for 1 min, 72°C for 2 min, and a final extension at 72°C for 10 min. The experiments were repeated three times and all produced similar results. The *OsActin* control primers were 5′- GGA ACT GGT ATG GTC AAG GC -3′ and 5′- AGT CTC ATG GAT ACC CGC AG -3′ [8].

Protein extraction and Western blot

T_1 generation seeds were frozen in liquid nitrogen and then ground to powder using a mortar and pestle. Total storage proteins in the rice endosperm were extracted with 50 mM Tris-HCl (pH 8.0) containing 2% SDS, 50% of 1-propanol and 1% of dithiothreitol, as described [4]. Amount of extracted total proteins was measured by Nanodrop Spec-

trophotometer (ND-1000, Thermo Fischer Scientific, Wilmington DE, USA). Western blot analysis was performed as described [22].

Simplified analysis of bread-making quality

The bread-making procedure used the straight dough method with 100 g of wheat and rice flours (Goami, Dongjin and *TaGlu-Ax1* transgenic rice), 6 g of sugar, 1.5 g of salt, 3 g of dry milk, 6 g of butter, 3 g of yeast and 60 g of fresh water at 30°C, 90% moisture conditions for 60 min first fermentation. After punching, second fermentation and baking were carried out at 30°C for 40 min, and 210°C for 25 min, respectively. Height of baked doughs were measured from bottom to top after baking.

Results

Vector construction and *Agrobacterium* transformation for marker-free transgenic rice

To improve dough properties of rice flour, we cloned high-molecule weight glutenin subunit gene (*TaGlu-Ax1*) from genomic DNA of *Triticum aestivum* cv. Jokyeong by PCR analysis with specific primers. We tried to generate marker-free transgenic plant expressing only *TaGlu-Ax1* gene on rice through *Agrobacterium*-mediated co- transformation system. To make a marker-free vector, we first removed the *HPTII* expression cassette (*CaMV 35S* promoter-*HPTII* gene- *CaMV 35S* terminator) by treatment of *XhoI* and *EcoRI* restriction enzymes and inserted wheat *Glu-Bx7*-own promoter. And then *TaGlu-Ax1* gene was constructed under the control of *Glu-Bx7*-own promoter into *pBTEX* vector, which was modified *pCAMBIA1300* binary vector (Fig. 1, upper panel). And original *pCAMBIA1300* binary vector harboring *HPTII* gene was used to select hygromycin resistant T_0 plants (Fig. 1, lower panel). The two expression binary vectors were separately introduced into *A. tumefaciens* EHA105 strain for plant transformation. Each binary vector was rescued from the EHA105 strain harboring *TaGlu-Ax1* and the *HPTII*, and the *HPTII* and *TaGlu-Ax1* genes were validated by PCR analysis.

Generation of marker-free *TaGlu-Ax1* transgenic rice plants

Each EHA105 strain harboring *TaGlu-Ax1* expression vector or *HPTII* expression vector was cultured in YEP medium for plant transformation. The cultured cells were resuspended to OD_{600} = 0.1 in AAM medium [14], and each *TaGlu-Ax1* and *HPTII* cell was added at a 3 : 1 ratio. These mixed cells were co-infected into rice calli. The transformed calli were selected with hygromycin because we co-infected calli with the *HPTII* gene. We obtained 210 independent hygromycin-resistant T_0 plants through co-infection in the *Agrobacterium* transformation system.

Genomic DNA from 210 independent T_0 plants was extracted and insertion of *HPTII* and *TaGlu-Ax1* genes was analyzed using PCR analysis with gene specific primers. As shown in Fig. 2, *HPTII* gene in all of T_0 plants was amplified, but no PCR products of Dongjin used as negative controls were detected. Next, we investigated the insertion of *TaGlu-Ax1* gene into rice genome within T_0 plants by PCR analysis. Among 210 independent transgenic lines, *TaGlu-Ax1* gene in 20 T_0 plants was amplified PCR product which is same

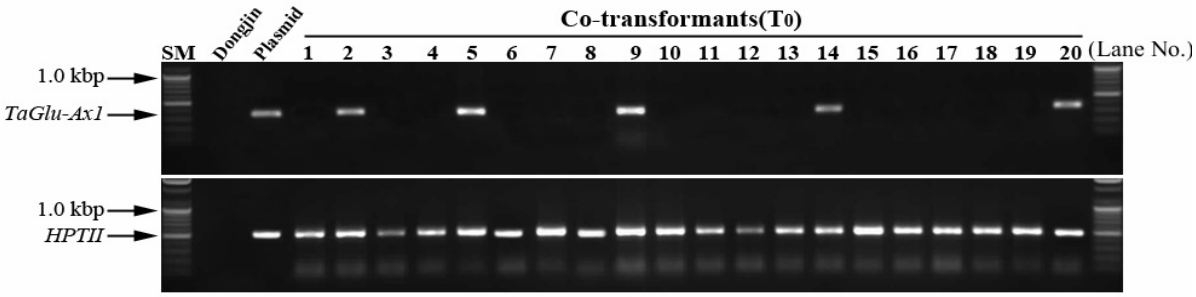

Fig. 2. Identification of T_0 plants by gene specific primer sets. *TaGlu-Ax1* (upper panel) and *HPTII* (lower panel) genes were amplified using *TaGlu-Ax1* and *HPTII* specific primer sets, respectively. SM, molecular marker; Dongjin (Korean rice cultivar), non-transgenic plant; Plasmid, vector construct containing *TaGlu-Ax1* and *HPTII* genes; 1-20, co-transformant transgenic lines, Lane No.; lane number. Only 5 transformants of 210 transfomants were shown in figure. PCR reactions were performed after randomly chosen 20 T_0 plants of 210 transformants (upper panel). Genomic DNAs from each plant were used as the template for *TaGlu-Ax1* and *HPTII* specific amplification. The reaction products of the sample plant were analyzed by electrophoresis on a 1.0% agarose gel.

Table 1. Co-transformation efficiency calculated during regeneration in rice-transformation experiments

Gene	No. of T_0 plants	No. of plants containing the *Glu-Ax1*	Frequency of co-transformation (%)
TaGlu-Ax1	210	20	9.52

PCR product size of plasmid used as positive controls (Fig. 2). This result means that 20 transgenic lines harbored both *TaGlu-Ax1* and *HPTII* genes. And the frequency co-transformation was about 9.52% in our experimental system (Table 1). We performed Southern blot analysis with the *TaGlu-Bx7*-own promoter as probes to validate their insertion and guessed segregation ratio of the marker-free plant in T_1 plants. One or multi signal bands in 20 selected T_0 plants lines were detected (Fig. 3).

Transcript and protein analysis of *TaGlu-Ax1* gene in the co-transformed rice plants

Because *TaGlu-Ax1* expression in rice endosperm is important for rice flour quality and we used *TaGlu-Bx7*-own promoter to express *TaGlu-Ax1*, total RNA from one copy-inserted T_1 generation transgenic seeds was extracted, and *TaGlu-Ax1* gene transcript level was examined by semi-quantitative RT-PCR after randomly chosen five T_1 seeds in line 6 of transformants. The *TaGlu-Ax1* transcripts were successively expressed in the T_1 generation transgenic seeds, whereas *TaGlu-Ax1* expression in Dongjin was not detected (Fig. 4). *OsActin* expression was used as a quantitative control. And we analyzed the protein expression of TaGlu-Ax1 by Western blot with an anti x-type HMW specific antibody. The seven transgenic plants (1, 2, 5, 9, 16, 18 and 20) which were shown abnormal morphologies comparing with Dongjin were removed. After total protein extraction from wheat ('Jokyeong' cultivar), Dongjin and transgenic plants (line 6 in fig. 3), 0.5 μg of wheat and 40 μg of total protein extract of transgenic plants were used for SDS-PAGE. The immunospecificity of the anti-x-type HMW specific antibody was verified by in vivo experiment. Although the protein bands were well detected in transgenic plants, however, the level of protein expression did not depend on their inserted copy number (Fig. 5). Multi- copies of *TaGlu-Ax1* gene were inserted into genome in some transgenic plants (16, and 20), no or weak bands were detected. We suppose that this phenomenon is homology-dependent gene silencing in plants [2].

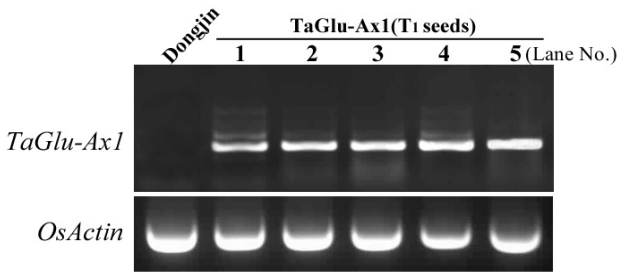

Fig. 4. Transcript analysis of the *TaGlu-Ax1* gene from T_1 seeds. RT-PCR was performed with *TaGlu-Ax1* T_1 seed transcripts to measure *TaGlu-Ax1* mRNA expression. Gene-specific PCR primers (forward and reverse primers) were designed to amplify the *TaGlu-Ax1* gene. *OsActin* was used as a control. RT-PCR reactions were performed after randomly chosen 5 T_1 seeds in line 6. The reaction products of the sample plant were analyzed by electrophoresis in a 1.0% agarose gel. Lane No.; lane number.

Selection of marker-free plants harboring *TaGlu-Ax1* gene in the T_1 generation

To select *TaGlu-Ax1* marker-free plants harboring only the *TaGlu-Ax1* gene, 100 T_1 generation seeds of the transgenic plant 6 were planted in soil and genomic DNA was extracted from leaves of plantlets after 4 weeks. Insertion of the *TaGlu-Ax1* and *HPTII* genes was investigated by PCR analysis. As shown in Fig. 6, most of the transgenic lines harbored both the *TaGlu-Ax1* and *HPTII* genes, and some inserted only the *HPTII* gene. However, transgenic 3, 4, 8 and 18 lines contained only the *TaGlu-Ax1* gene (Fig. 6). This result shows

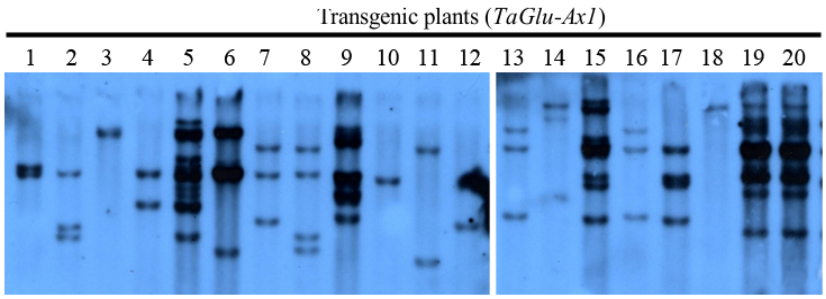

Fig. 3. Southern hybridization analysis of *TaGlu-Ax1* gene from T_0 plants. The 1.35 kb fragment of Ta*Glu-Bx7*-own promoter was amplified by PCR using specific primer sets as the probe.

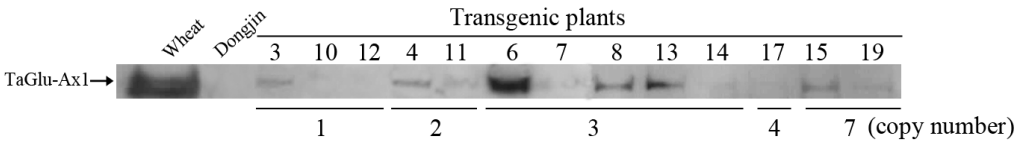

Fig. 5. Protein expression analysis of *TaGlu-Ax1* gene from T_1 seeds. Western blotting was performed with an anti x-type HMW specific antibody. Total protein extracts of 0.5 μg of wheat and 40 μg of transgenic plants and 'Dongjin' were used for SDS-PAGE. The numbers were indicated tansformants lines.

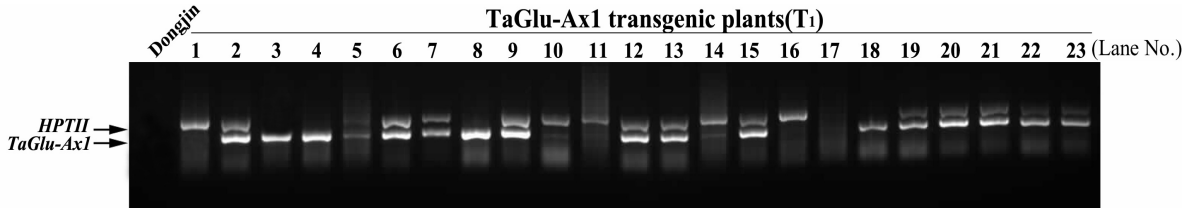

Fig. 6. PCR analysis of T_1 progenies to select marker-free transgenic plant containing *TaGlu-Ax1* gene. Dongjin, non-transgenic plant as negative control; 1-23, T_1 progeny lines from T_0 plants containing both *TaGlu-Ax1* and *HPTII* genes, Lane No.; lane number. PCR reactions were performed after randomly chosen 23 T_1 plants in line 6. The reaction products of the sample plant were analyzed by electrophoresis in a 1.0% agarose gel.

that marker-free plants containing only the *TaGlu-Ax1* gene were successfully screened at the T_1 generation. Finally, we produced marker-free transgenic rice plants harboring *TaGlu-Ax1* gene for advanced quality processing of bread and noodle.

Analysis of bread-making quality in marker-free transgenic rice

In this study, we developed marker-free *TaGlu-Ax1* transgenic rice. The final objective of this study is to develop transgenic rice of increased processing quality than non-transgenic rice. Therefore, we first measured swollen ratio of dough in *TaGlu-Ax1* transgenic rice (line 6), with Jokyeong (Korea wheat variety), Dongjin and Goami (Korea rice variety) which used for material of rice noodle after first fermentation. As a result, the marker-free *TaGlu-Ax1* transgenic rice was slightly higher than Goami on swollen ratio of dough, whereas the Dongjin showed same level with the marker-free *TaGlu-Ax1* transgenic rice (Fig. 7). This results indicated that only one HMW-GS (*TaGlu-Ax1*) insufficient for increasing processing quality (especially bread-making quality) of transgenic rice dough. Finally, we think that accumulation and combination of more HMW-GSs and LMW-GSs and gliadins of wheat is required for improved processing quality of rice dough.

Discussion

Wheat gluten proteins are classified into two broad groups based on their aggregation and functional properties, including the glutenins, which form polymers stabilized by inter-chain disulfide bonds, and gliadins, which are present as monomers and interact by non-covalent forces [29]. In particular, HMW-GSs are minor components in terms of quantity, but they are key factors during bread making because they are major determinants of gluten elasticity [30] by promoting the formation of larger glutenin polymers.

In this study, we cloned *TaGlu-Ax1*, which is one of HMW-GS genes, and validated the insertion of the *TaGlu-*

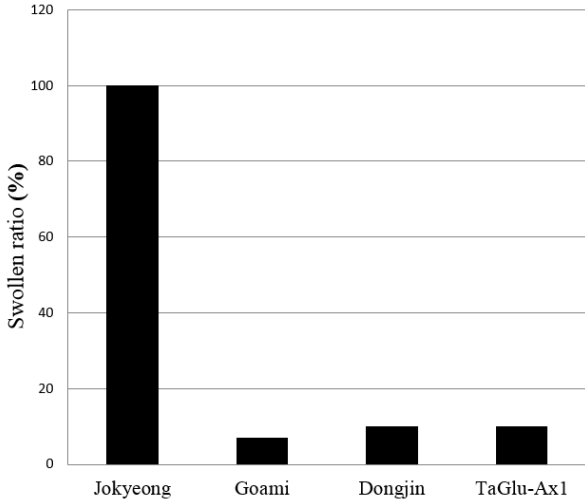

Fig. 7. Analysis of bread-making quality of Jokeong (Korea wheat variety), Goami (Korea rice variety), Dongjin (non-transgenic rice) and marker-free transgenic rice (T_2 seeds in line 6). Heights of swollen doughs of Jokyeong and rice flours were measured after baking procedure.

Ax1 gene in T_0 plants through PCR analysis with gene specific primers and Southern blot analysis (Fig. 2, Fig. 3). The *TaGlu- Bx7*-own promoter was introduced for seed specific expression of *TaGlu-Ax1* gene.

The transcript and protein of *TaGlu-Ax1* in the transgenic plants were stably expressed in T_1 generation rice seeds (Fig. 4). This result suggests that the protein processing system was conserved between rice and wheat. However, the level of protein expression did not depend on their inserted copy number (Fig. 5). Genetic engineering of plants sometimes results in transgene silencing after integration into the genome, which may relate to a defense mechanism against foreign DNA expression [18, 32]. This phenomenon may be related to homology-dependent gene silencing in plants. Homology-dependent gene silencing has attracted considerable interest because it may be detrimental to genetic engineering and also because of its usefulness as a tool to study the mechanisms involved in detecting and inactivating exogenous DNA [18, 20].

Multi- copies of *TaGlu-Ax1* gene were inserted into genome in some transgenic plants (5, 9, 19 and 20), no proteins were detected. We suppose that this phenomenon is homology-dependent gene silencing in plants [2].

The co-transformation frequency in our experimental conditions was 9.52% (Table 1). In a previous report, co-transformation frequency in rice was about from 2% to 14% [16]. This result indicates that transformation efficiency is dependent on rice cultivar and the experimental conditions. Although the generating of marker-free plants based on the *Agrobacterium*-mediated co-transformation using two different expression cassettes was need more time consuming and effort, this method could be efficiently produced marker-free transgenic rice plants. In addition, expression of only the *TaGlu-Ax1* gene in rice was no better the processing quality (Fig. 7). However, co-expression of *TaGlu-Ax1* and *PINA* in durum wheat is increased dough strength and resistance to extension than lines expressing *TaGlu-Ax1* or *Pina* [19]. Accumulation and combination of more HMW-GSs and LMW-GSs, gliadins and other genes of wheat is required for improved processing quality of rice dough.

As a result, the marker-free *TaGlu-Ax1* transgenic rice developed in this study is valuable to be utilized in breeding programs as a good material for improved processing quality.

Finally, we obtained marker-free transgenic plants containing only *TaGlu-Ax1* gene from each of the T_1 plants (Fig. 6). This marker-free transgenic plant harboring *TaGlu-Ax1* will become useful material to optimize transgenic rice plants, which has advanced quality processing of bread and noodle by crossing with genetically engineered rice plants with other gluten genes. In other words, our results should be seen as a process for improving the processing quality.

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초록 : 벼에서 밀 고분자 글루테닌 단백질(*TaGlu-Ax1*) 발현을 통하여 쌀가루 가공적성 증진을 위한 마커프리(marker-free) 형질전환 벼의 개발

정남희[1†] · 전승호[2†] · 김둘이[1] · 이춘석[1] · 옥현충[1] · 박기도[1] · 홍하철[1] · 이승식[3] · 문중경[4] · 박수권[1*]
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밀의 고분자 글루테닌 서브유닛[high molecular-weight glutenin subunit (HMW-GS)]은 밀가루의 성질을 결정하는데 가장 중요한 요소이며 가공적성을 나타내는데 중요한 역할을 수행한다. 우리는 *Agrobacterium* 동시 형질전환법을 이용하여 한국 밀 품종인 '조경'으로부터 밀 HMW-GS을 암호화하는 *TaGlu-Ax1* 유전자를 가지는 marker-free 형질전환 벼를 생산하였다. *TaGlu-Ax1* 유전자의 종자 특이적 발현을 위하여 밀에서 존재하는 *TaGlu-Bx7* 유전자의 자체 프로모터를 벡터 내에 삽입하였다. 동시 접종을 위해서 오직 *TaGlu-Ax1* 유전자와 hygromycin phosphotransferase II (*HPTII*) 저항성 유전자만으로 구성된 두 종류의 발현 카세트를 독립적으로 *Agrobacterium* EHA105에 도입하였고, *TaGlu-Ax1*와 *HPTII*가 도입된 각각의 EHA105 *Agrobacterium*을 3:1 비율로 혼합하여 벼 캘러스에 접종하였다. 210개의 *HPTII* 저항성 형질전환체 중에서 벼 게놈에 *TaGlu-Ax1*과 *HPTII*가 모두 삽입된 20개의 형질전환 라인을 획득하였다. *TaGlu-Ax1*와 *HPTII*가 벼 게놈에 도입된 것을 Southern blot을 통해서 다시 확인하였다. 형질전환 벼 T_1 세대의 종자에서 밀 *TaGlu-Ax1* 유전자가 전사와 번역되어 오직 *TaGlu-Ax1*만을 가지는 marker-free 식물체를 T_1세대에서 성공적으로 선발할 수 있었다. *TaGlu-Ax1* 유전자가 발현되는 marker-free 형질전환 식물체는 야생형(wild type)과의 표현형 차이는 없었다. 형질전환 벼의 쌀가루의 제빵적성을 비교하였을 때 *TaGlu-Ax1* 유전자만이 발현되어서는 제빵적성이 더 나아지지 않았다. 그러므로 더 많은 밀 고분자 및 저분자 글루테닌, 글리아딘의 유전자의 집적과 조합이 쌀가루 가공적성을 증진시키는데 필요하다. 결론적으로 *TaGlu-Ax1* marker-free 형질전환 벼는 쌀가루 가공적성을 증진시키는데 좋은 재료로 사용될 것이다.