Overexpression of a Chromatin Architecture-Controlling *ATPG7* has Positive Effect on Yield Components in Transgenic Soybean

Hye Jeong Kim^{1†}, Hyun Suk Cho^{1†}, Jun Hun Pak¹, Kook Jin Kim², Dong Hee Lee², Young-Soo Chung^{1*}

¹Department of Molecular Genetics, College of Natural Resources and LifeScience, Dong-A University, Busan 49315, Korea

²Genomine Advanced Biotechnology Research Institute, Genomine Inc., Pohang 37668, Korea

ABSTRACT AT-hook proteins of plant have shown to be involved in growth and development through the modification of chromatin architecture to co-regulate transcription of genes. Recently, many genes encoding AT-hook protein have been identified and their involvement in senescence delay is investigated. In this study, soybean transgenic plants overexpressing chromatin architecture-controlling *ATPG7* gene was produced by *Agrobacterium*-mediated transformation and investigated for the positive effect on the important agronomic traits mainly focusing on yield-related components. A total of 27 transgenic soybean plants were produced from about 400 explants. T₁ seeds were harvested from all transgenic plants. In the analysis of genomic DNAs from soybean transformants, *ATPG7* and *Bar* fragments were amplified as expected, 975 bp and 408 bp in size, respectively. And also exact gene expression was confirmed by reverse transcriptase-PCR (RT-PCR) from transgenic line #6, #7 and #8. In a field evaluation of yield components of *ATPG7* transgenic plants (T₃), higher plant height, more of pod number and greater average total seed weight were observed with statistical significance. The results of this study indicate that the introduction of *ATPG7* gene in soybean may have the positive effect on yield components.

Keywords Soybean, *ATPG7*, *Agrobacterium*-mediated transformation, Transgenic plants, Yield component

INTRODUCTION

AT-hook proteins of plant have shown to be involved in growth and development through the modification of chromatin architecture to co-regulate transcription of genes (Su *et al.* 2006; Lim *et al.* 2007; Street *et al.* 2008; Xiao *et al.* 2009). Many AT-hook motif proteins were identified from a variety of species such as *Arabidopsis*, maize and rice in plants (Li *et al.* 2002). The modulation of chromatin architecture is induced by genes controlling DNA methylation and histone acetylation/deacetylation and functional genes of chromatin binding. This chromatin architecture plays an important role in growth and development in plants including meristem identity, leaf longevity and the development of flowering and seed formation (Su *et al.*

2006; Lim *et al.* 2007; Street *et al.* 2008; Xiao *et al.* 2009).

Recently, many genes related to senescence delay have been identified in a research team of Genomine Inc. (data not shown). Several genes encoded for AT-hook binding proteins have been investigated for their function in chromatin remodeling and subsequent senescence delay in *Arabidopsis* (unpublished data). Functional change of *Arabidopsis* in morphology due to senescence delay resulted in increased biomass and yield.

Soybean (*Glycine max* [L.] Merr.), as a major crop, is important for the production of vegetable oil and protein in worldwide. Many reports point to the beneficial effects of soybean on human health and even as a source for medicines (Oksman-Caldentey and Hiltunen 1996; Zeng *et al.* 2004; Manavalan *et al.* 2009; John *et al.* 2016).

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*Corresponding author Young-Soo Chung, chungys@dau.ac.kr, Tel: +82-51-200-7510, Fax: +82-51-200-6536

† These authors contributed equally.

Soybean transformation has mostly been developed with *Agrobacterium*-mediated transformation (Hinchey *et al.* 1988), and improved rapidly by the use of half-seed explants (Paz *et al.* 2006). In our previous study (Kim *et al.* 2012), Soybean transformation efficiency was consistently improved with additional treatments such as addition of thiol compounds, sonication and vacuum. The improved efficiency made us enable to produce enough stable transgenic soybean plants with a few transformation trials. Ten to twenty healthy plants could be obtained from 300–400 seeds of explants. This improvement has substantial implication as a big forward step to functional genomics of soybean via transgenic research. With this modified transformation protocol, many genes have been introduced to soybean in order to investigate their physiological change or improvement in crops (Kim *et al.* 2013; Kim *et al.* 2016; Kim *et al.* 2017).

In this study, we generated soybean transgenic plants overexpressing chromatin architecture-controlling *ATPG7* gene by *Agrobacterium*-mediated transformation and investigated the important agronomic traits mainly focusing on yield-related components.

MATERIALS AND METHODS

Vector construction and *Agrobacterium* preparation

ATPG7 (AT-hook protein of Genomine 7) gene (GenBank: DI237375.1) was provided by Dr. D.H. Lee (Genomine Inc., Korea) and was amplified by PCR using *ATPG7*-F primer (5'- ATGGATCCAGTACAATCTCATGGA-3') and *ATPG7*-R primer (5'- TCAATACG-TGGTTCGTCCTCCGTTGA-3'). The desired destination vector, pCSEN (provided by Dr. D.H. Lee) was used for the vector construction. The plasmid, pCSEN-*ATPG7* was constructed and transformed into *Agrobacterium tumefaciens* strain EHA105 (Karimi *et al.* 2002), following the protocol described by Kim *et al.* (2013, 2016, 2017).

Soybean transformation

Korean cultivar soybean seeds (*Glycine max* L. cv. Kwangankong) were utilized in *Agrobacterium*-mediated transformation experiments, following the protocol des-

cribed by Kim *et al.* (2013, 2016, 2017). T₀ plants with two trifoliates were screened using an herbicide assay to determine the resistance and to identify putative transformants that express *Bar* gene. The upper surface of a leaf was painted with 100 mg/L PPT using a brush. The response to the herbicide was observed at 3–5 days after PPT leaf painting. The PPT-resistant T₀ plants were then grown in a greenhouse, and T₁ seeds were harvested.

Genomic DNA analysis of transgenic plants

Total genomic DNA was extracted from leaf tissues of non-transgenic (NT) and T₀ soybean plants using the cetyltrimethylammonium bromide. The polymerase chain reaction (PCR) was performed to detect the introduced genes with KOD FX polymerase (TOYOBO, Japan) according to the manufacturer's instructions, and two primer sets were designed to amplify regions of *ATPG7* (5'-ATG-GATCCAGTACAATCTCATGGA-3'/5'-TCAATA-CGGTGGTTCGTCCTCCGTTGA-3') and *Bar* (5'-TCCGT-ACCGAGCCGCAGGAA-3'/5'-CCGGCAGGCTGAA-GTCCAGC-3'). PCR was performed using KOD FX polymerase (TOYOBO, Osaka, Japan) according to the manufacturer's instructions.

RNA analysis of transgenic plants

Total RNAs were isolated from NT and T₀ soybean plants using Plant RNA Purification Reagent (Invitrogen, USA) according to the manufacturer's instructions. Reverse transcriptase-PCR (RT-PCR) was performed using Maxime RT-PCR Premix (iNtRon, Korea) according to the manufacturer's instructions. The primer pairs used in the RT-PCR were as follows: *ATPG7* forward, 5'-ATG-GATCCAGTACAATCTCATGGA-3'/reverse, 5'-TCA-ATACGGTGGTTCGTCCTCCGTTGA-3'; *Bar* forward, 5'-TCCGTACCGAGCCGCAGGAA-3'/reverse, 5'-CCG-GCAGGCTGAAGTCCAGC-3'. The constitutively expressed gene *TUB* was used as a control and amplified using the primer set 5'-TGAGCAGTTCACGGCCA-TGCT-3'/5'-TCATCCTCGGCAGTGGCATCCT-3').

Investigation of yield components in GMO field

NT and transgenic soybean seeds (T₃ generation) were planted in a seedling tray, and treated with 100 mg/L PPT

for the herbicide assay. Seedlings were then transplanted in GMO field (Keunwi, Kyungsangbukdo) in order to evaluate yield components including plant height, the number of branches per plant and the number of nodes per plant. Moreover, the number of pods per plant and the total seed weight were also determined to investigate the relative yield of transgenic plants.

RESULTS

Production of transgenic soybean plants via *Agrobacterium*-mediated transformation

To produce transgenic soybean plants with high-yield, the corresponding pCSEN-*ATPG7* plasmid (Fig. 1) was used for the soybean transformation with half-seeds of the Korean soybean cultivar Kwangankong (Fig. 2) described by Kim *et al.* (2013, 2016, 2017). A total of 27 transgenic soybean plants were produced from about 400 explants. T₁

seeds were harvested from all transgenic plants. The whole procedure, starting from seed preparation to T₁ seed harvest, has taken about 6-7 months. Further generations were proceeded until T₃ generation in GMO field (Keunwi, Kyungsangbukdo).

Fig. 1. Vector used for soybean transformation. Schematic representation of the vector pCSEN-*ATPG7* containing *ATPG7* and *Bar* genes used for soybean transformation. The *Bgl*II and *Bst*EII restriction enzyme sites are also marked. LB/RB: left/right T-DNA border sequences, p35S/T35S: CaMV (cauliflower mosaic virus) 35S promoter/terminator, pSEN: stress-inducible promoter, *Bar*: coding region of the DL-phosphinothricin resistance gene.

Fig. 2. Production of soybean transgenic plants with *ATPG7* gene using *Agrobacterium*-mediated transformation. (a) Co-cultivation of half-seed explants after infection (left) and at five days after inoculation (right); (b) Shoot induction medium without PPT; (c) Shoot induction medium containing 10 mg/L PPT for *Bar* selection. (d) Shoot elongation medium containing 5 mg/L PPT; (e) Rooting medium; (f) Acclimation of putative transgenic plant in a small pot; (g) Transgenic plant (T₀) grown in a large pot in a greenhouse; (h) Leaf painting with herbicide (100 mg/L PPT) to reveal the sensitivity of non-transgenic plant (left) and resistant of transgenic plant (right).

Analysis of transgene integration and expression

To confirm the integration of the transgene in soybean transformants, genomic DNAs were isolated from T_0 transformants, and PCR was carried out to analyze the presence of *ATPG7* and *Bar* genes, respectively (Data not shown). Only healthy and well-grown transgenic line #6, #7 and #8 were selected for the investigation of agronomic characteristics, and these lines were examined using PCR with *ATPG7* and *Bar* primers to amplify the DNA fragments of 975 bp and 408 bp in size, respectively (Fig. 3a). To analyze the transcript levels of *ATPG7* and *Bar*, reverse transcriptase-PCR (RT-PCR) was carried out with RNAs extracted from transgenic line #6, #7 and #8. As shown in Fig. 3b, these genes were expressed in all 3 lines tested.

Investigation of yield components in transgenic soybean

To examine the yield components of *ATPG7* transgenic

Fig. 3. Analysis of transgene integration and expression in *ATPG7* transgenic plants (T_0). (a) Confirmation of introduced genes in *ATPG7* transgenic plants (T_0) using PCR. Genomic DNAs were extracted from T_0 transgenic leaf tissues. (b) *ATPG7* and *Bar* gene expression in T_0 transgenic plants using reverse transcriptase-PCR (RT-PCR). Total RNAs were extracted from T_0 plants, and then analyzed by RT-PCR with the *TUB* gene as a quantitative control. NT: non-transgenic plant, #6-8: *ATPG7* transgenic lines (T_0).

plants (T_3), we compared their agronomic characteristics to those of NT plants in GMO field. Agronomic traits including plant height, the number of nodes per plant, branches per plant, pods per plant and total seed weight of plants and transgenic lines (#6, #7 and #8) were investigated (Fig. 4). The plant height of transgenic lines was similar to, or higher than NT plants. The number of pods in transgenic lines was more than those of NT plants, as showing about 16%, 38% and 40% increase in line #6, #7 and #8, respectively. In total seed weight, transgenic line #6, #7 and #8 showed approximately 15%, 60% and 40% greater than non-transgenic plants with statistical significance ($P < 0.05$ in line #6 and $P < 0.01$ in line #7 and #8). This result is significantly related to the yield increase. Therefore, the important yield components of transgenic line #6, #7 and #8 were more favorable for high yield than those of normal wild-type plants.

DISCUSSION

Relatively large number of stable transgenic plants was obtained in this experiment. We have been observed the transformation efficiency varied by different genes and constructs. The production of 27 plants out of 400 explants is relatively high frequency. The result might be from the additional treatments. Before we used the modified method, average efficiency of transformation was less than 3% (unpublished data). Our *Agrobacterium*-mediated soybean transformation was primarily established based on the cotyledonary-node (CN) method. Then half-seed method was mainly used for recent transformation experiment. The later method skipped the germination procedure of soybean seed on germination media. The biggest advantage of half-seed method is saving at least one week in the early procedure of transformation and no requirement for sophisticated wounding on explant. This modified protocol enabled us to produce the successful soybean transgenic plants with many genes (Kim *et al.* 2012; Kim *et al.* 2013, Kim *et al.* 2016; Kim *et al.* 2017).

Many chromatin architecture-controlling genes via senescence delay, which created phenotypic change including organ size of plants and yield components, have

Fig. 4. Agronomic characteristics of T₃ transgenic plants in GMO field. NT and T₃ *ATPG7* transgenic plants (n=20 for each line) were grown in GMO field, and agronomic traits including plant height (a), the number of branches and nodes per plant (b), the number of pods per plant (c) and total seed weight (d) were investigated. Error bars indicate mean±standard deviation. Asterisks indicate significant changes compared with NT (**P*<0.05; ***P*<0.01). NT: non-transgenic plants, #6-8: *ATPG7* transgenic lines (T₀).

been discovered by a research team of Genomine Inc. They obtained the result on the basic function of these genes with transgenic research in model plant, and hope to see similar result in crop like soybean. It may be too early to say the positive effect of those genes in the yield components of soybean with our limited results. Further generation should be evaluated for their yield-related main agronomic characters and connection with gene expression. The year of 2017, we have deployed next generation of 3 lines (#6, #7 and #8) to investigate the correlation between field performance of transgenic plants and gene expression at the cell level. The current result suggests that the overexpression of *ATPG7* gene in soybean contribute to the positive effect on yield components, indicating the modification of senescence could lead to yield increase.

Considering the daily accumulating new genes from genomic research, transgenic research based on high-efficient transformation will have an important role in many respects. More active trial with transgenic crop should be carried with new candidate genes.

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REFERENCES

- Hinchee MAW, Connor-Ward DV, Newell CA, McDonnell RE, Sato SJ, Gasser CS, *et al.* 1988. Production of transgenic soybean plants using *Agrobacterium*-mediated DNA transfer. *Nat. Biotechnol.* 6: 915-922.
- John KMM, Natarajan S, Luthria DL. 2016. Metabolite changes in nine different soybean varieties grown under field and greenhouse conditions. *Food Chem.* 211: 347-355.
- Karimi M, Inze' D, Depicker A. 2002. Gateway™ vectors for *Agrobacterium*-mediated plant transformation. *Trends Plant Sci.* 7: 193-195.
- Kim HJ, Kim MJ, Pak JH, Im HH, Lee DH, Kim KH, *et al.* 2016. RNAi-mediated soybean mosaic virus (SMV) resistance of a Korean soybean cultivar. *Plant Biotechnol. Rep.* 10: 257-267.
- Kim HJ, Kim MJ, Pak JH, Jung HW, Choi HK, Lee YH, *et al.* 2013. Characterization of SMV resistance of soybean produced by genetic transformation of *SMV-CP* gene in RNAi. *Plant Biotechnol. Rep.* 7: 425-433.
- Kim MJ, Kim HJ, Pak JH, Cho HS, Choi HK, Jung HW, *et al.* 2017. Overexpression of *AtSZF2* from *Arabidopsis* showed enhanced tolerance to salt stress in soybean. *Plant Breed. Biotech.* 5: 1-15.
- Kim MJ, Kim JK, Kim HJ, Pak JH, Lee JH, Kim DH, *et al.* 2012. Genetic modification of the soybean to enhance the β -carotene content through seed-specific expression. *PLoS One* 7: e48287.
- Li G, Hall TC, Holmes-Davis R. 2002. Plant chromatin: development and gene control. *BioEssays* 24: 234-243.
- Lim PO, Kim Y, Breeze E, Koo JC, Woo HR, Ryu JS, *et al.* 2007. Overexpression of a chromatin architecture-controlling AT-hook protein extends leaf longevity and increases the post-harvest storage life of plants. *Plant J.* 52: 1140-1153.
- Manavalan LP, Guttikonda SK, Tran LSP, Nguyen HT. 2009. Physiological and molecular approaches to improve drought resistance in soybean. *Plant Cell Physiol.* 50: 1260-1276.
- Oksman-Caldentey KM, Hiltunen R. 1996. Transgenic crops for improved pharmaceutical products. *Field Crops Res.* 45: 57-69.
- Paz MM, Martinez JC, Kalvig AB, Fonger TM, Wang K. 2006. Improved cotyledonary node method using an alternative explant derived from mature seed for efficient *Agrobacterium*-mediated soybean transformation. *Plant Cell Rep.* 25: 206-213.
- Sreet IH, Shah PK, Smith AM, Avery N, Neff MM. 2008. The AT-hook-containing proteins SOB3/AHL29 and ESC/AHL27 are negative modulators of hypocotyl growth in *Arabidopsis*. *Plant J.* 54: 1-14.
- Su Y, Kwon CS, Bezhani S, Huvermann B, Chen C, Peragine A, *et al.* 2006. The N-terminal ATPase AT-hook-containing region of the *Arabidopsis* chromatin-remodeling protein SPLAYED is sufficient for biological activity. *Plant J.* 46: 685-699.
- Xiao C, Chen F, Yu X, Lin C, Fu YF. 2009. Over-expression of an AT-hook gene, AHL22, delays flowering and inhibits the elongation of the hypocotyl in *Arabidopsis thaliana*. *Plant Mol. Biol.* 71: 39-50.
- Zeng P, Vadnais DA, Zhang Z, Polacco JC. 2004. Refined glufosinate selection in *Agrobacterium*-mediated transformation of soybean [*Glycine max* (L.) Merrill]. *Plant Cell Rep.* 22: 478-482.