Design of Construct Carrying GmDREB6 to Enhance Soybean Gene Expression Related to Abiotic Stress Response

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Abstract—Glycine max (L.) Merrill is a crop that brings a lot of economic and nutritive values, however soybean is quite sensitive to stress. The applications of gene technology can improve resistance of soybean plants against external stress factors. The aim of this study was to conduct a transgenic vector containing GmDREB6 gene and determine the expression of gene encoding GmDREB6 in Nicotiana tabacum before transforming into soybean plants. The GmDREB6 artificial gene was synthesized containing nucleotide fragment encoding 230 amino acids, nucleotide fragment encoding emyc antigen and nucleotide fragments with cut-off points of Xbal/SacI enzyme pair. The results indicated that the 35S-GmDREB6 vector construct was designed and transferred into tobacco plants. The GmDREB6 was incorporated in the genome and was expressed in transgenic tobacco plants at the transcriptional level. The transgenic vector pBI121_GmDREB6 well worked on the model tobacco plants. Therefore, it can be used for transferring into soybean plants to enhance soybean tolerance to abiotic stress.

Index Terms—Abiotic Stress, Agrobacterium-Mediated Transformation, GmDREB6 Gene, Transcription Factors, Transgenic Vector.

I. INTRODUCTION

Soybean (Glycine max (L.) Merrill) not only brings economic, nutritive value but also can improve soil fertility. Soybean is considered as a crop that is quite sensitive to adverse impacts from externalities. Biotic (such as virus, bacteria and fungi) and abiotic (like drought, salinity, heat shock) stress have seriously affected the growth, development, productivity and quality of soybean. Some studies on improvement biotic and abiotic stress tolerance of crops and soybeans using gene transfer have been reported [1]-[5]. In 2015, Lo et al. generated a GmEXP1 construct to express recombinant expansion protein in tobacco plants. It was observed that overexpression of the GmEXP1 gene in transgenic tobacco plants. The transgenic tobacco plants have improved drought tolerance as a result of an increase of the length and volume of transgenic plant roots in comparison to non-transgenic plants [1]. The 35S-GmDREB2-cmyc construct carrying GmDREB2 gene was designed and transformed into Nicotiana tabacum [2]. According to these authors, the proline accumulation of the transgenic tobacco lines was higher than on non-transgenic plants during drought conditions. DREB (dehydration-responsive element binding) transcription factor has been identified and its function is to activate genes involved in abiotic stress response. The cis-element of DREB gene and the trans-factor of DREB protein play an important role in gene expression to react to abiotic stress. Trans-factors bind to cis-elements to activate expression of functional genes in plants under stressed conditions. In soybean, it was detected that GmDREB6 gene has locus LOC100101914 on chromosome 5, the CDS region is 693 bp in length which encodes 230 amino acids. DNA-binding domain in plant proteins includes AP2 and EREBP. AP2 region of DREB protein play an important role in abiotic stress response. The GmDREB6 gene and determine the expression of this gene expression to react to abiotic stress. Trans-factors bind to cis-elements to activate expression of functional genes in plants under stressed conditions.
transcription factor GmDREB6 in the transgenic soybean plants.

II. MATERIALS AND METHODS

A. Materials

The tobacco plants, K326, were used as the model plant for evaluation of the construct carrying GmDREB6 gene. pBI121 transgenic vector; the enzymes XbaI, SacI; Agrobacterium tumefaciens CV58. pBI121 transgenic vector contains neomycin-phosphotransferase II gene (nptII) and cauliflower mosaic virus 35S promoter (35S promoter).

B. Generating GmDREB6 transgenic construct and recombinant A. tumefaciens

Transgenic vector carrying GmDREB6 gene was designed following two basic steps: (1) design of independent structure including GmDREB6 gene, cmyc segment and the cutting positions of XbaI/SacI enzyme pair (GmDREB6-cmyc); (2) insert the structure into plant transgenic vector, pBI121 to form recombinant vector pBI121_GmDREB6.

Recombinant vector was transferred into A. tumefaciens by electrical pulses (2.5 kV, 25 μF, 200 Ω) to create recombinant A. tumefaciens.

C. Agrobacterium-mediated transformation

Agrobacterium-mediated transformation via leaf infection and regeneration of tobacco plants was done as previously described by Topping (1998) [8]. Tobacco leaves were cut into 1×1 cm pieces, soaked in cell suspension of recombinant A. tumefaciens in 10 mins, and then regenerated multi-shoots on MS medium with added BAP and kanamycin. The shoots transferred into RM medium with added kanamycin for root generation. The plantlets were grown in greenhouses.

D. Analysis of transgenic tobacco plants

Total DNA from tobacco leaves was extracted according to method of Saghai-Marooif et al. (1984) [9] using the Trizol Reagents Kit (Invitrogen). cDNA was synthesized from RNA using Maxima® First Strand cDNA Synthesis Kit (Fermantas).

GmDREB6 transgene was amplified from transgenic tobacco plants by PCR and transcription of GmDREB6 transgene was confirmed by RT-PCR. The primer pair, XbaI-DREB6-F/ DREB6-SacI-R is used for PCR.

XbaI-DREB6-F:
5'- ATGAAAGTTCAACCAACCACCTCTCAT-3'
DREB6-SacI-R:
5' -ATTCAAGATCTCTTCTAGATGAGT-3'.

Incorporation of GmDREB6 transgene in the genome of tobacco plants was confirmed by Southern blot analysis (Southern 1975) [10]. The GmDREB6 gene was amplified using PCR with the primer pair XbaI-DREB6-F/ DREB6-SacI-R. The probe DNA was marked with a Biotin Labeling Kit DNA DecaLabel using biotin-11-dUTP. Genomic DNA samples from the PCR-positive transgenic plants in T0 generation were cut by restriction enzyme SacI at 37°C. DNA fragments cut by SacI enzyme were evaluated by agarose gel electrophoresis and then DNA fragments were transferred from electrophoresis gel to the hybrid membrane and implement hybrid with probe DNA. The membrane was hybridized overnight at 44°C. The hybridization result was displayed on X-ray film.

Expression of GmDREB6 gene at the transcriptional level was analysed by RT-PCR with primer pair XbaI-DREB6-F/ DREB6-SacI-R.

III. RESULTS AND DISCUSSION

A. Construction of transgenic vector carrying GmDREB6 gene

Based on information of GmDREB6 (cDNA) isolated from DT2008 Vietnamese soybean cultivar and GmDREB6 with EF551166 code on GenBank (Liu et al., 2007) [6], artificial GmDREB6 gene was designed with 741 bp in size and nucleotide sequence of GmDREB6 gene as follows:

GmDREB6

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In which 5' - gctctaga is the sequence contains the cutting position of XbaI enzyme and gagctcg is the sequence contains the cutting position of SacI enzyme.

Gene GmDREB6 was structured by 693 bp coding region, 8 bp (GCTCTAGA) at 5’ end (the cutting position of XbaI) and 7 bp (GAGCTCG) enzymes in 3’ end (the cutting position of the enzyme SacI). A segment of 33 bp which encodes the cmyc antigen at the 3’ end was added. Compared with the sequence of GmDREB6 isolated from soybean DT2008 and sequence of GmDREB6 with the code EF551166 on GenBank, the artificial GmDREB6 sequence has G replaced by T at position 417.

GmDREB6 gene was inserted to pUC18 vector. Transgenic vector pBI121 contained GUS gene. XbaI/SacI enzyme pair was used to cut and remove GUS gene from pBI121_GUS, and then insert GmDREB6 transgene into pBI121 transgenic vector, creating construct transgenic probe pBI121_GmDREB6 (Fig 1).

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Fig. 1. Design diagram of transgenic vector pBI121_GmDREB6. A: pBI121_GUS vector; B: pUC18_GmDREB6 vector; C: pBI121_GmDREB6; nptII: neomycin-phospho-transferase II; 35S: cauliflower mosaic virus 35S promoter; GmDREB6::cmyc: GmDREB6 gene contains cmyc and cutting positions of the XbaI / SacI enzyme pair.

The results of cutting pUC18-GmDREB6 to obtain GmDREB6 gene and removing GUS gene from vector pBI121_GUS were shown in Figure 2A. Recombinant vector pBI121_GmDREB6 which was transferred into E.coli DH5α, was cloned and tested by colony-PCR (Fig. 2B).

Plasmid pBI121_GmDREB6 extracted from E.coli DH5α was transferred into A.tumefaciens. The recombinant A. tumefaciens carrying GmDREB6 gene was cloned and selected (Fig. 3). The positive colonies of A.tumefaciens was used to infect into tobacco.

Fig. 2. A- Electrophoresis gel of cutting products from the pPU18_GmDREB6 and pBI121_GUS vector with SacI XbaI enzyme pair. 1: pPU18_GmDREB6; M: DNA marker; 2: pBI121_GUS. B- Electrophoresis gel of colony-PCR product from E.coli DH5α colonies to test GmDREB6 gene in pBI121_GmDREB6 construct. M: DNA marker; (+): GmDREB6 gene amplified from pPU18_GmDREB6; 1, 2, 3, 4: GmDREB6 gene amplified from E.coli DH5α colonies.

Fig. 3. Electrophoresis gel of colony-PCR products from A.tumefaciens AGL1 colonies to test GmDREB6 transgene. M: DNA marker; 1, 2, 3, 4: the colony lines of A.tumefaciens strains AGL1.
B. Transferring the pBI121_GmDREB6 construct into tobacco plants

Pieces of tobacco leaf that were about 1cm² in size were cultured on MS media for 48 hours; after that, the leaf pieces were immersed in the A.tumefaciens suspension in 20 minutes. The bacterial infected leaf pieces were transferred to the co-cultivation medium, the multi-shoot regeneration medium, the rooting medium respectively, to create transgenic tobacco plants (Fig.4).

![Fig. 4. Results of creating transgenic tobacco plants by infecting combinant through leaf pieces. A: The leaf pieces in the A.tumefaciens suspension. B: The transformed samples were transferred to the co-cultivation medium; C, D: Samples on multi-shoot regeneration medium and the shoot elongation medium; E: The shoots on the rooting medium; (F): The transgenic tobacco plants grown on substrates in the greenhouse.](image1)

From 180 samples in three independent experiments, 539 shoots were induced on multi-shoot regeneration and shoot elongation media supplemented with kanamycin, in which 309 elongated shoots were rooted. And 101 rooted plantlets were planted on the pots, among which 48 plantlets were transferred to the greenhouse. Thirteen transgenic plants which have well grown and developed, were analyzed to determine the presence of the GmDREB6 transgene. The results of PCR analysis with XbaI-DREB6-F/ DREB6-SacI-R primer pair showed that nine positive plants with the emergence of a DNA band in the same size of GmDREB6 (741 bp) (Fig. 5A). The PCR- positive transgenic plants in the T0 generation were labeled as T0-4, T0-5, T0-6, T0-7, T0-8, T0-9, T0-11, T0-12, T0-13.

The results of the Southern blot analysis showed that among the nine PCR-positive plants, there were eight plants (T0-4, T0-5, T0-6, T0-7, T0-8, T0-9, T0-11, T0-12, T0-13) that had positive results of the Southern blot (Fig. 5B). Consequently, GmDREB6 transgene has been incorporated in the genome of transgenic tobacco plants.

![Fig. 5. GmDREB6 in the transgenic tobacco plants confirmed by PCR (A) and Southern blot (B) analyses. M: DNA marker; (+): plasmid pBI121-GmDREB6; WT: non-transgenic plants; 1-13 (A) and 1-9 (B): T0 generation transgenic plants as T0-4, T0-5, T0-6, T0-7, T0-8, T0-9, T0-11, T0-12, T0-13.](image2)

Results of transcriptional analysis of eight T0 plants positive to Southern blot showed that there are five T0 plants were transcribed to generate mRNA including T0-5, T0-7, T0-9, T0-12, T0-13 (Fig. 6). Therefore, GmDREB6 transgene was expressed in tobacco plants.

![Fig. 6. Results of transcriptional analysis of eight T0 plants positive to Southern blot showed that there are five T0 plants were transcribed to generate mRNA including T0-5, T0-7, T0-9, T0-12, T0-13.](image3)

IV. CONCLUSION

The GmDREB6 gene was designed with the size of 741 bp encoded 230 amino acids and the nucleotide segment encodes the cmyc antigen used to test the presence of recombinant GmDREB6 protein in transgenic plants. The 35S-GmDREB6-cmyc construct in recombinant vector pBI121 was designed and transferred into tobacco plants by Agrobacterium-mediated transformation. The GmDREB6 transgene was incorporated in the genome and expressed in...
transgenic tobacco plants at the transcriptional level. The transgenic vector pBI121_GmDREB6 well worked on the model tobacco plants, so it can be used to transfer into target plants improves abiotic stress tolerance.

Fig. 6. The RT-PCR products of GmDREB6 transgene amplified from mRNA of the T0 transgenic plants. M: DNA marker; (+): plasmid pBI121-GmDREB6; WT: non-transgenic plants; 1, 2, 3, 4, 6, 7, 8, 9: The transgenic tobacco plants were positive to Southern blot, T0-4, T0-5, T0-6, T0-7, T0-9, T0-11, T0-12, T0-13 respectively.

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CONFLICT OF INTEREST
The authors declare that there are no conflicts of interest.

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