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Characterization of the *GmDREB5* gene isolated from the soybean cultivar Xanh Tiendai, Vietnam

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***Abstract*— Soybean (*Glycine max* (L.) Merrill) belongs to the crop group which has low drought tolerance. In recent years, droughts have occurred more and more commonly as a result of climate change. Therefore, it is necessary to study on genes involved in drought tolerance of soybean. The dehydration-responsive element binding (DREB) protein is a transcription factor activating the gene expression in the drought stress signaling pathway of plants in general and soybean in particular. In this study, we present some results on amplification of *GmDREB5* gene from mRNA isolated from soybean cultivar Xanh Tiendai in Vietnam via RT-PCR reaction using specific primers DREB5soyF/DREB5soyR, and cloning and sequencing this gene. This gene is 924 bp in length, including 207 Ts, 268 As, 190 Gs and 259 Cs, with the similarity reached 90.4% compared with the sequence of *GmDREB5* gene (Accession No. EF583447) in the GenBank of a Chinese soybean cultivar. Amino acid sequence of the polypeptide encoded by these two genes are 87.8% similar. There are, however, differences appeared in some positions of nucleotide and amino acid sequences between this two genes and two polypeptides. This study is the basis for later studies on GmDREB5 gene, such as investigation of the function of this gene in the resistance of soybean plants to drought stress.**

Keywords-soybean; Glycine max; GmDREB5 gene; transcription factor; drought tolerance; RT-PCR

I. INTRODUCTION

Soybean (*Glycine max* (L.) Merrill), a species of legume, not only have high economic and nutritional value but only play an important role in improving soil fertility and sustainable use of cultivated land resources. In recent years, droughts have occurred more and more commonly as a result of global warming and climate change. This has affected the growth of plants productivity of crops. Soybean belongs to the crop group which has low drought tolerance. Therefore, selective breeding high drought tolerance soybean cultivars and studying on methods to improve the drought tolerance of soybean are interesting topics of many scientists.

A number of genes have been described that respond to drought stress at the transcriptional level [5]. The *cis*-acting elements and *trans*-acting factors play significant roles in drought responsive gene expression [4]. In 1994, Yamaguchi-Shinozaki and Shinozaki identified a *cis*-acting dehydration-responsive element (DRE), which is present in the promoter of *COR78/RD29A* and involved in response to drought, low temperature, and high salt stresses. The *trans*-acting factor, DRE-binding (DREB) protein can bind to DRE to activate the gene expression in the stress-signaling pathway of plants [7]. DREB protein is a subfamily of AP2/ERF transcription factors, which control expression of many drought, salinity and cold inducible genes and causing tolerance to environmental stresses in many plants. The main property of DREB gene is conserved AP2 domain that binds to stress responsive elements [1]. Many DREB genes have been isolated from various plants, such as *Arabidosis*, rice (*Oryza sativa* L.), maize (*Zea mays* L.), wheat (*Triticum aestivum*) and many other crops [1,3,4].

At least 10 members of the DREB gene subfamily are present in the soybean genome (*GmDREBa, GmDREBb, GmDREBc, GmDREB1, GmDREB2, GmDREB3, GmDREB5, GmDREB6, GmDREB7*) [6]. These genes play important roles in the resistance of soybean plants to drought stress by recognizing the dehydration responsive element. Among these genes, there are few studies on *GmDREB5*. To date, there was only one report (Chen et al, 2007) about isolation of *GmDREB5* gene from soybean with 927 bp in length [2]. In this study, we present some results on amplification and characterization of *GmDREB5* gene from mRNA isolated from soybean cultivar Xanh Tiendai in Vietnam. This study is the basis for later studies on GmDREB5 gene, such as investigation of the function of this gene in the resistance of soybean plants to drought stress.

II. MATERIALS AND METHODS

Plant materials: *Glycine max* cultivar Xanh Tiendai, Vietnam was supplied by Vietnam Academy of Agricultural Sciences (2009).

Cloning of the *GmDREB5* gene: Total RNA was isolated using AccuZol™ Total RNA Extraction Reagent (Bioneer) and used for cDNA reverse transcription using RevertAid™ H Minus First Strand cDNA Synthesis Kit

(Fermentas). The cDNA was used as template for PCR with a gene-specific primer pair Dreb5soyF: 5'-ATGCAATTCCCTCACCAATT-3' (forward); Dreb5soyR: 5'-TCAATCCTGATCCTTCCACA-3' (reverse).

PCR products were analyzed via electrophoresis in 1% agarose gel and purified using GeneJET™ Gel Extraction Kit (Fermentas). After that, it were inserted into pBT vector and transformed into *E. coli* DH5α competent cells. After checking colonies via colony PCR, plasmids were extracted using AccuPrep Plasmid Extraction Kit (Bioneer).

Sequencing: Nucleotide sequence of *GmDREB5* gene was determined by using ABI PRISM 3100-Avant Genetic Analyzer with BigDye Terminator v 3.0 kit.

III. Results and Discussion

A. Cloning of the GmDREB5 gene

Total RNA was isolated from the gemmae of soybean cultivar Xanh Tiendai, Vietnam and used for cDNA reverse transcription using RevertAid™ H Minus First Strand cDNA Synthesis Kit (Fermentas). The cDNA was used as template for PCR with a gene-specific primer pair Dreb5soyF/Dreb5soyR. These primers were designed based on the sequence of GmDREB5 gene published in NCBI GenBank (Accession No. EF583447) [2].

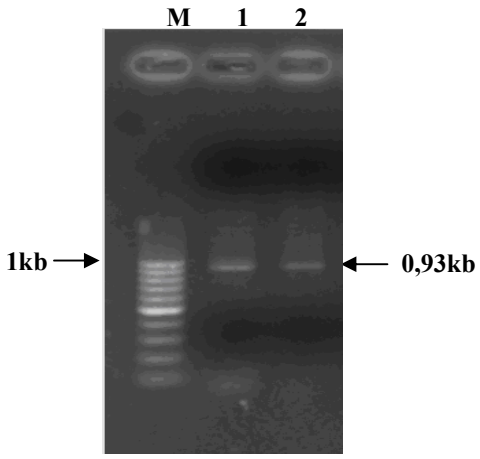

Figure 1. Electrophoresis of RT-PCR products (M: marker; 1&2: RT-PCR products)

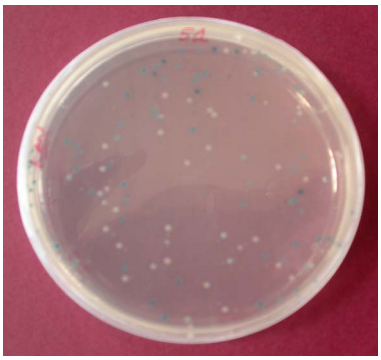

Figure 2. A LB agar plate showing the result of a blue white screen

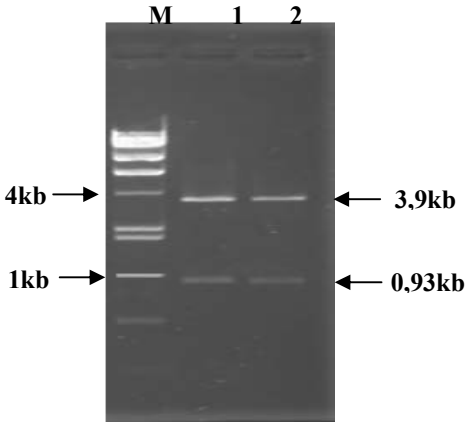

Figure 3. Electrophoresis of the Restriction enzyme reaction products (M: marker; 1&2: restriction enzyme reaction products)

The PCR products were analyzed via electrophoresis in agarose gel (Fig. 1). Both two samples showed a band approximately 0.93 kb in size. This size is suitable with theoretical calculation and similar to the size of *GmDREB5* gene published in GenBank (0.927kb). Therefore, we decided to use this PCR product for transforming and cloning.

PCR product was inserted into pBT vector and transformed into *E. coli* DH5α competent cells. These bacteria were cultured in LB agar medium supplemented with kanamycin and X-gal and incubated for 16 hours. The result was shown in Fig. 2.

Several white colonies were selected to cultured in LB medium for 16 hours, shaking at 200 rpm in 37^0C. Plasmids were extracted and cleaved by EcoRI restriction enzyme, checked via electrophoresis (Fig. 3).

B. Sequencing of the GmDREB5 gene and comparing with the sequence of the GmDREB5 gene (Accession No. EF583447) isolated from a Chinese soybean cultivar

Sequencing of the *GmDREB5* gene and comparing with the sequence of the *GmDREB5* gene (Accession No. EF583447) isolated from a Chinese soybean cultivar. After purified, recombinant plasmids were used for nucleotide sequencing. The *GmDREB5* gene of Xanh TienDai cultivar includes 207 Ts, 268 As, 190 Gs and 259 Cs (Fig. 4).

The sequences of the *GmDREB5* gene isolated from Xanh Tiendai cultivar and a Chinese soybean cultivar (Accession No. EF583447) [2] are 90.4 % similar. The nucleotide sequence of Xanh Tiendai cultivar doesn't have nucleotides at the positions 193-202 (AGCACCA GC) and 527-535 (AAGA ACAAC) compared with the gene accession no. EF583447. The nucleotide sequence of the *GmDREB5* gene accession no. EF583447 doesn't have nucleotides at the positions 605-607 (CCC), 675-677 (GGT) and 882-890 (TGATGCTCA).

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                 ....|....| ....|....| ....|....| ....|....| ....|....|
                         10         20         30         40         50
EF 583447        ATGCAATTCC CTCACCAATT TGGAACCACA ACAAACTCAC CTTTCCCTCA
Xanhtiendai      ATGCAATTCC CTCACCAATT TGGAACCACA ACAAACTCAC CTTTCCCTCA
                 ....|....| ....|....| ....|....| ....|....| ....|....|
                         60         70         80         90        100
EF 583447        CCCATCTTTC CAAAACCAGC AACACCAGAT GATATCATTT GGGTCTTCCC
Xanhtiendai      CCCATCTTTC CAAAACCAGC AACACCAGAT GATATCATTT GGGTCTTCCC
                 ....|....| ....|....| ....|....| ....|....| ....|....|
                        110        120        130        140        150
EF 583447        AACAACATAA TAATCTCGCA TATCCACCCA TCATAGCCAG TGATTCTTCT
Xanhtiendai      AACAACATAA CAATCTCGCA TATCCACCAA TCATAGCCCG AGATTCTTCT
                 ....|....| ....|....| ....|....| ....|....| ....|....|
                        160        170        180        190        200
EF 583447        TCACTTCTAC ATCAACATCA TCATCATCAA CAGCAGCATC AGCAGCACCA
Xanhtiendai      TCACTTGTAC ATCATCATCA TCAACAACAA CAACAGCATC AGC-------  <-
                 ....|....| ....|....| ....|....| ....|....| ....|....|
                        210        220        230        240        250

EF 583447        GCAGCAACTT CTTCAGTATT GGAGTGACGC GTTGAATCTA AGTCCAAGAG
Xanhtiendai      --AGCAACTT CTTCAGTATT GGAGTGGCGC GTTGAATCTA AGTCCAAGAG  <-

                 ....|....| ....|....| ....|....| ....|....| ....|....|
                        260        270        280        290        300
EF 583447        GAATGTTAAC AAGATTGGGA CCAGATGGAA GGCCATTGTT TAGGCTTCCA
Xanhtiendai      GAATGTTAAC AAGGTTGGGG CCAGATGGAA GGCCATTGTT TAGTCCTCCA
                 ....|....| ....|....| ....|....| ....|....| ....|....|
                        310        320        330        340        350
EF 583447        ACACAGCCCA TAAACACAAC AAAACTCTAT AGAGGAGTGA GGCAACGCCA
Xanhtiendai      ACACAGCGCA TAAACACAAC AAAACTCTAT AGGGGAGTGA GGCAACGCCA
                 ....|....| ....|....| ....|....| ....|....| ....|....|
                        360        370        380        390        400
EF 583447        TTGGGGGAAA TGGGTCGCTG AAATCCGTCT TCCACGAAAC AGAACGCGTC
Xanhtiendai      TTGGGGCAAA TGGGTCGCTG AAATCCGTCT TCCACGAAAC AGAACGCGTC
                 ....|....| ....|....| ....|....| ....|....| ....|....|
                        410        420        430        440        450
EF 583447        TCTGGCTAGG CACATTTGAC ACGGCCGAAG ACGCCGCCAT GGCCTACGAC
Xanhtiendai      TCTGGCTAGG CACATTTGAC ACGGCCGAAG ACGCCGCCAT GGCCTACGAC
                 ....|....| ....|....| ....|....| ....|....| ....|....|
                        460        470        480        490        500
EF 583447        CGAGAAGCCT TCAAGCTACG AGGAGAGAAT GCTAGACTCA ATTTCCCAGA
Xanhtiendai      CGCGAAGCCT TCAAGCAACG AGGAGAGAAT GCAAGGCTCA ATTTCCCCGA
                 ....|....| ....|....| ....|....| ....|....| ....|....|
                        510        520        530        540        550
EF 583447        ATTGTTCCTC AACAAGGACA AAAAAGAAGA ACAACAACAA CAAGAACAAG
Xanhtiendai      ATTGTTCTTC AACAAGGACA AAAAAG---- -----AACAA GGAGAAGAAG  <-
                 ....|....| ....|....| ....|....| ....|....| ....|....|
                        560        570        580        590        600
EF 583447        AAGCTTCTTC GCCAGTTCTT TCAGCTATTG CAAAGCAGCA TGAGCCTTCT
Xanhtiendai      AAGCTTCTTC GCCGGTTCTT TCGGCTATTG CAAAGCAGAA TGAACCTCCT
                 ....|....| ....|....| ....|....| ....|....| ....|....|
                        610        620        630        640        650
EF 583447        AGTG---AAC ACCGTGACGT CCCCATAGAA GAGTCTAATG AGAATGATTC  <-
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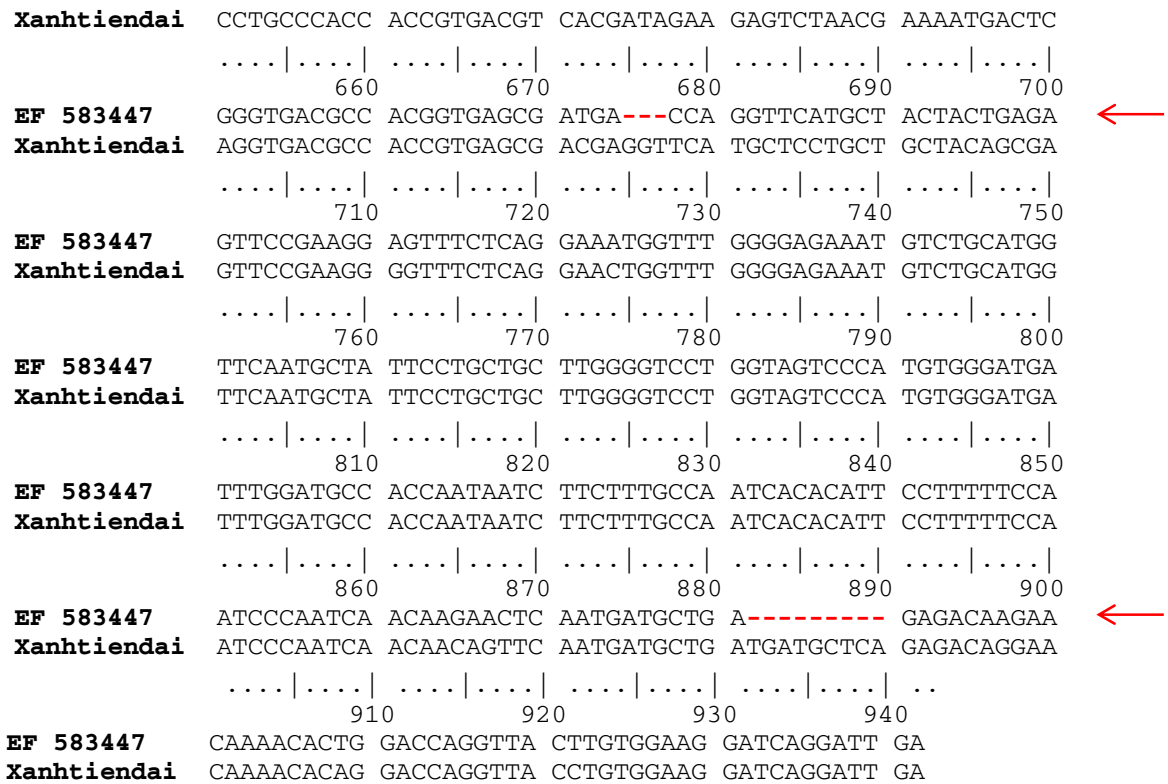
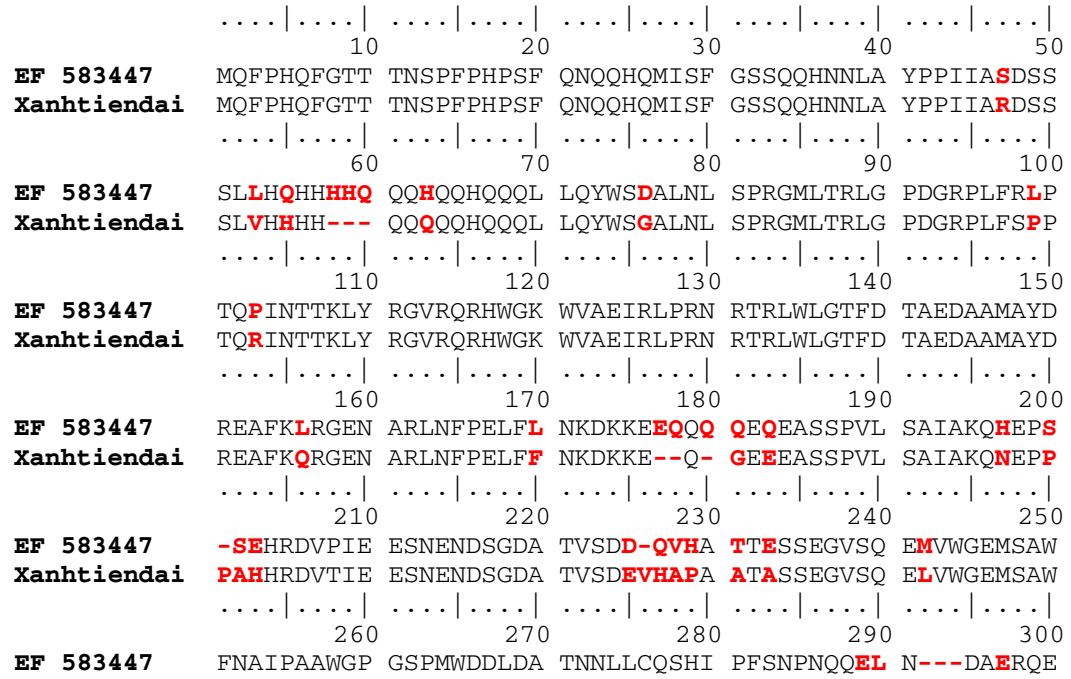


Figure 4. Comparison of the nucleotide sequences of the *GmDREB5* gen isolated from soybean cultivar Xanh Tiendai, Vietnam and the *GmDREB5* gene accession no. È583447



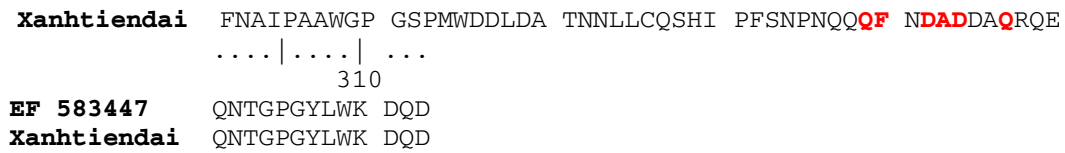


Figure 5. Comparison of the amino acid sequences predicted from *GmDREB5* gen of soybean cultivar Xanh Tiendai, Vietnam and from the *GmDREB5* gene accession no. È583447

We also compared the amino acid sequence predicted from the nucleotide sequence of the *GmDREB5* gene of these two cultivars (Fig. 5). These two amino acid sequences are 87.8% similar. The amino acid sequence of Xanh Tiendai cultivar doesn't have amino acids at positions 58 – 60 (HHQ) and positions 177, 178 and 180 (EQQ). The amino acid sequence of the Chinese soybean cultivar doesn't have amino acids at positions 201 (P), and 292- 294 (DAD).

IV. CONCLUSION

We have designed and synthesized the gene-specific primer pair DREB5soyF/DREB5soyR, successfully cloning the *GmDREB5* gene from RNA isolated of soybean cultivar Xanh Tiendai, Vietnam. The nucleotide sequence of this gene includes 207 Ts, 268 As, 190 Gs and 259 Cs. The nucleotide sequences of *GmDREB5* gene isolated from Xanh Tiendai cultivar and a Chinese soybean cultivar are 90.4 %, and the amino acid sequences predicted from the *GmDREB5* gene of these two cultivars are 87.8%. There are some differences at several positions between these two nucleotide and amino acid sequences. It is necessary to have next studies on comparison of the sequences of the *GmDREB5* gene isolated from low drought tolerant soybean cultivars and the ones from high drought tolerant cultivars.

ACKNOWLEDGMENT

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