Cloning and Designing Vector Carrying *GmEXP1* Gene Isolated from Local Soybean Cultivar Sonla, Vietnam

Lo Thanh Son, Le Van Son, Nguyen Vu Thanh Thanh, and Chu Hoang Mau

***Abstract*—Soybeans (Glycine max (L.) Merrill) are short-duration industrial crops which have high economic and nutritional values, and play an important role in improving soil fertility and sustainable use of cultivated land resources. Soybeans have low level of drought tolerance, thus it is of great necessity to study on approaches to improve their drought tolerance, including using genes related to the root elongation. The *GmEXP1* gene which expresses expansin, a key protein in cell expansion, is one of those genes. In this study, we amplified, cloned and determined the *GmEXP1* gene sequence from local soybean SL1 cultivar with best vigorous root systems. *GmEXP1* gene is 790 bp in length, encoding 255 amino acids. Transgenic vector carrying *GmEXP1* gene has been designed successfully (pCB301-*GmEXP1)* and transformed into tobacco plants (*N. tabacum* K326). These results form the basis for generation of transgenic soybean cultivars overexpressing *GmEXP1*, aiming to improve the drought tolerance based on the root elongation of soybean cultivars in Vietnam.**

***Index Terms*—Expansin, *GmEXP1* gene, gene transfer, local soybean, root elongation.**

I. Introduction

Soybeans (*Glycine max* (L.) Merrill) are short-duration industrial crops which have high economic and nutritional value, as well as play an important role in improving soil fertility and sustainable use of cultivated land resources.. In recent years, the global warming and climate change affected the growth of soybean cultivars in Vietnam. Soybean belongs to the crops group which has low drought tolerance, thus the application of biotechnology to improve the drought tolerance of soybean has become interesting topics of Vietnamese scientists.

Two major mechanisms related to drought tolerance of plants is the osmotic pressure adjustment and the development of a strong root systems. Root system plays an important role in providing water to get to the life and activities of plant development.

The drought tolerant mechanism of plants closely related to the development of the root systems. Deeper rooting should improve soybean's ability to avoid water stress by increasing water uptake from deep soil layers [1]. During growing process, plant cells secrete a protein called expansin, which unlocks the network of wall polysaccharides. Expansin's action has puzzling implications for plant cell-wall structure. The recent explosion of gene sequences and expression data has given new hints of additional biological functions for expansins [2]. Expansins are cell wall proteins, containing two known families, named alpha- and beta-expansins, and they comprise large multigene families whose members show diverse organ-, tissue- and cell-specific expression patterns [3]. Expansins belong to a group of extracellular proteins that directly modify the mechanical properties of plant cell walls, leading to turgor-driven cell extension. Based on phylogenetic analysis, the studies have shown that the three plant expansin subfamilies (α, β và γ-expansin) arose and began diversifying very early in, colonization of land by plants [4].

Expansins are plant proteins that can induce extension of isolated cell walls and are proposed to mediate cell expansion and is considered a major protein affects the expansion of the plant cell [5]. Lee *et al*. (2003) isolated expansin genes (EXP1) from mRNA of soybean and found that the expression levels of *GmEXP1* were very high in the roots of 1- to 5-d-old seedlings, in which rapid root elongation takes place [6]. Furthermore, *GmEXP1* mRNA was most abundant in the root tip region, where cell elongation occurs, but scarce in the region of maturation. The *GmEXP1* gene plays an important role in root development in soybean, especially in the elongation and initiation of the primary and secondary roots [6]. Among the local soybean cultivars in Vietnam, cultivar SL1 was judged to be the most vigorous root systems. Thus we used cultivar SL1 for the isolation of *GmEXP1* gene with the aim of creating materials for gene transfer to improve drought tolerance of soybean crops.

In this study, we present some results on amplification of *GmEXP1* gene from mRNA isolated from soybean cultivar SL1 in Vietnam, designing a plant expression vector carrying *GmEXP1* structure and transferred it into tobacco cultivars (*Nicotiana tabacum*).

II. Materials and Methods

Local soybean cultivar Sonla (SL1) in Vietnam was supplied by Vietnam Academy of Agricultural Sciences (2013). Tobaco cultivars *N. tabacum* K326 was supplied by Department of Plant Cell Technology, Institute of Biotechnology, Vietnam.

Total RNA was isolated using Trizol Reagent KIT, cDNA was synthesized using the Maxima ® First Strand cDNA Synthesis KIT. The cDNA was used as template for PCR to

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amplified encoding region of *GmEXP1* gene. From the information about sequence of *GmEXP1* gene in soybean on GenBank, code number AF516879, we designed primer pair SoyExpF/SoyExpR for the isolation of gene encoding region of *GmEXP1* gene:

SoyExpF: CATGCCATGGATGGGCAAAATCATGCTTGT
SoyExpR:ATTTGCGGCCGCTTAGAACTGAACTGGGCTAGA

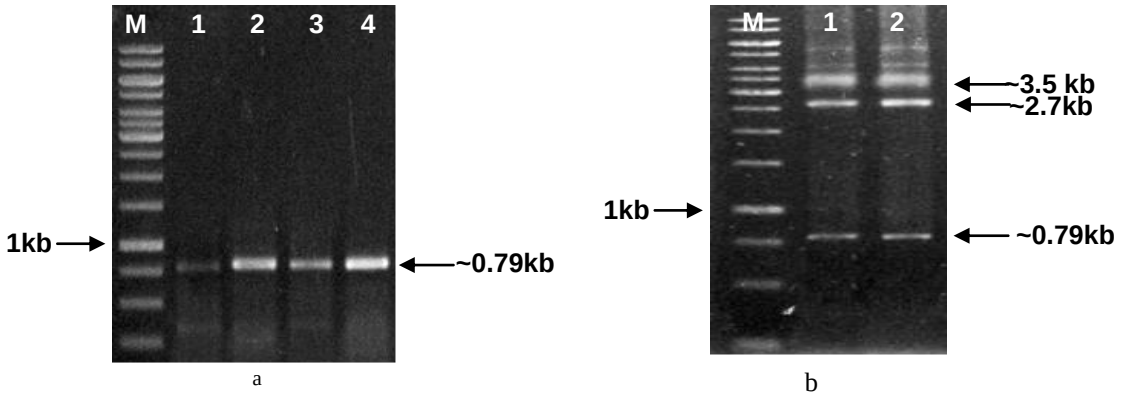
PCR products were examined by electrophoresis on a 0.8% agarose gel and purified by JET^{TM} Gene Gel Extraction KIT; recombinant vector transformed into *E. coli* DH5α cells with variable heat shock. Bacteria carrying recombinant vector were selected on antibiotic selective environment (carbenicilin, kanamycin). The strain carrying the recombinant vector were selected by colony-PCR with specific primers, were cultured in liquid LB supplemented environment antibiotic selection appropriate to collect biomass. Recombinant plasmids were obtained by extracting the molecular cloning methods [7].

To transfer *GmEXP1* structure into plants and be able to check protein expression, we designed pCB301 recombinant vector carrying the target gene structure consists of the following components: CaMV35S_*GmEXP1*_c-myc_KDEL_polyA. Recombinant vector transferred into *Agrobacterium tumefaciens* cells by electrical pulses (2.5 kV, 25 μF, 200 Ω). The bacteria carrying the recombinant gene structure is multiplied with large biomass and used to infect into tobacco plants.

III. Results and Discussion

A. Cloning and Sequence Determining of GmEXP1 Gene from Soybean Cultivar SL1

From cultivar SL1, we extracted total RNA from the grown domain of roots and synthesized cDNA with random primers (random hexamer primers). Coding region of *GmEXP1* gene was amplified by PCR with specific primer pair (SoyExpF/SoyExpR) and RT-PCR products obtained a very special brand with length approximately 0.79 kb as expected. (Fig. 1 a). PCR products were purified and inserted into cloning vector pBT_2705bp and transformed into *E. coli DH5α*. We selected clones carrying recombinant plasmid, extracted recombinant plasmids and checked the size of the insert by *BamH*I. The results confirmed that plasmid carrying an insert with expected size (Fig. 1 b).



a b

Fig. 1. a) PCR products of the *GmEXP1* gene (cDNA) amplifed from soybean cultivar SL1 and b) Products from plasmid digestion by *BamH*I (M: DNA Marker 1kb).

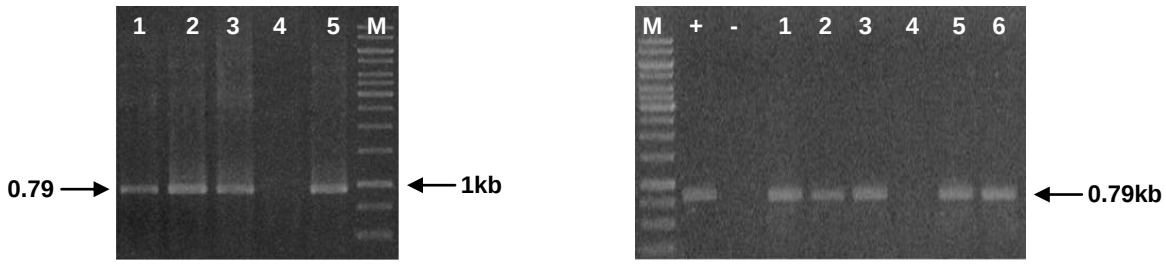
The results of gene sequencing showed that *GmEXP1* gene is 790 bp in length, encoding 255 amino acids. Results comparing with gene sequences *GmEXP1* published on GenBank by using BLAST in NCBI show that *GmEXP1* gene sequence isolated from soybean cultivar SL1 is similar to that in *GmEXP1* gene sequence (code number: AF516879) on Genbank with 99% of similar coefficients (only different on 5 nucleotides and 1 amino acid). This result confirmed that we have successfully cloned *GmEXP1* gene from the soybean cultivar SL1 and *GmEXP1* gene sequence has been registered on GenBank with code is HG792075.

B. Construction of Transgenic Vector Carrying GmEXP1 Gene

Vector pBT carrying *GmEXP1* gene was digested by two restriction enzymes *Nco*I and *Not*I to cut out GmEXP1 gene (DNA fragments with the size of 0.79 kb). Gen *GmEXP1* was then inserted into vector pRTRA to create the recombinant structure carrying the transgene (pRTRA-*GmEXP1*). Recombinant vector pRTRA-*GmEXP1* was transformed into *E. coli* DH5α cells. The transformed *E. coli* cells were selected and cultured in LB environment supplement with carbenicilin. Extracted plasmids were then tested for the presence of *GmEXP1* gene by PCR with specific primer pair SoyExpF/SoyExpR (Fig. 2 a).

Recombinant vector pRTRA_*GmEXP1* was cut by *Hind*III to obtain two DNA fragments with the approximate size of 1.6kb and 2.3kb, in which the 1.6 kb DNA fragment contains our target gene (CaMV35S_*GmEXP1*_c-myc_KDEL_polyA). Structure CaMV35S_*GmEXP1*_c-myc_KDEL_polyA was inserted in vector pCB301 to create transgenic vector carrying the transgene (pCB301_CaMV35S_*GmEXP1*_c-myc_KDEL_polyA). This vector was cloned into *E. coli* DH5α. We then used colony-PCR with specific primer pair SoyExpF/SoyExpR to select colonies having *GmEXP1* gene (Fig. 2 b). Plasmid pCB301_CaMV35S_*GmEXP1*_c-myc_KDEL_polyA is

extracted from positive colonies and transformed into *A.tumefaciens* in order to transform into tobacco plants.



a b

Fig. 2. a) PCR products of the *GmEXP1* gene amplified from pETRA-*GmEXP1* on gel agarose 0.8%; b) Colony-PCR products of the *GmEXP1* gene from colony lines (+: positive control; -: negative control; 1, 2, 3, 4, 5, 6: colony lines).

TABLE I: TRANSFORMATION AND REGERATION OF TOBACCO PLANTS CARRYING PCB301_CAMV35S_GMEXP1_C-MYC_KDEL_POLYA STRUCTURE

No.	Number of gene transfer samples	Number of samples create buds	Number of buds on medium for generated roots	Number of trees planted in pots
1	30	30	88	74
2	30	29	87	75
3	40	38	102	93
Sum	*100*	*97*	*277*	*242*

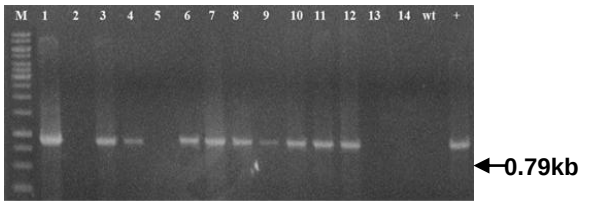


Fig. 3. PCR detection for the presence of transgene in transgenic tobacco plants (M: DNA marker 1kb; +: positive control; wt: non-transgenic tobacco plants; 1-14: transgenic tobacco lines).

C. The Results Transformants Structure PCB301-GmEXP1into Tobaco Cultivars (N.tabacum K326)

Leaves of tobacco plants (*N. tabacum* K326) were cut into 1 × 1 cm pieces, soaked in cell suspension of recombinant *A.tumefaciens* in 10 minutes, and then regenerated on MS medium with added BAP. The well grown buds (2 - 3 cm) were excised and transferred into RM medium for root generation. The plantlets were grown in greenhouses. The results throughout the transformation and regeneration process are presented in Table I.

We have obtained 242 tobacco lines in total from 100 transformed samples. Analyses of 44 tobacco cultivars by PCR to check for presence of *GmEXP1* gene in the transgenic plants and the results showed that 32/44 regenerated plants tested positive for the PCR reaction (see Fig. 3).

Expansins have an important role in the phase of cell growth of root systems of soybean cultivars. Most of the experiments showed that at tissue containing growing cells, expansins altering the physical properties of the cell wall by loosening hydrogen bonding, loosening bonding non-chemotherapy between micro fiber of cellulose and loosening of polymer network [8]. Micro fibers of cellulose are connected together by the polysaccharide. The polysaccharide linked to surface of micro-fibers of cellulose and bonded together. Expansin protein can break the polysaccharide bonds of micro-fiber surface or break the bonds between the polysaccharide and the distance between the micro fibers of cell walls easily be pushed stretch both horizontally and vertically. Expansin can move easily between the micro-fibers of cellulose of cell walls and in contact with the polymer adhesive points. This facilitates the growth phase of cells to be easily done [2]. Expansin have more in the epidermal cells and in the cell layer at growth domain of roots and the lateral roots, but very rare in the mature domain [6]. Soybeans is group of plants have drought tolerance with low level, so approach studying the of elements impact on the root elongation in soybean plants forms the basis of the application of gene transfer technique to improve drought tolerance of soybean plants. Along with the successful transformants of the *GmEXP1* gene isolated from soybean cultivars into tobacco plants, it can be said this is the basis to be able to create transgenic soybean cultivars carrying structure pCB301-*GmEXP1*.

IV. CONCLUSION

GmEXP1 gene has been isolated and cloned from local soybean cultivar SL1 (Sonla, Vietnam). *GmEXP1* gene has the size of 790 bp, encoding 255 amino acids. Transgenic vector carrying *GmEXP1* gene has been designed successfully and transformed into tobacco plants (*N. tabacum* K326). This work forms the basis for the generation of transgenic soybean cultivars carrying structure pCB301-*GmEXP1*, aiming to improve the drought tolerance soybean cultivars in Vietnam by using approaches to develop their root system.

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