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Designing Primers with a Plant Signal Peptide to Enhance the Expression of *GBA1* in Transgenic Soybean Plants

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Abstract:

Transgenic plants offer advantages for the manufacture of recombinant proteins with terminal mannose residues on their glycan chains. So plants are chosen as source of pharmaceutical products and for the development of alternative expression systems to produce recombinant lysosomal enzymes. In the present study the sequence of the natural cDNA encoding for the human lysosomal enzyme glucocerebrosidase (GCD) was modified to enhance its expression in soybean plants. The glucocerebrosidase gene signal peptide was substituted with that signal peptide for the *Arabidopsis thaliana* basic endochitinase gene to support the co-translational translocation into the endoplasmic reticulum (ER), and the storage vacuole. So, targeting signal from tobacco chitinase A, to facilitate GCD trafficking from the ER to the storage vacuole, appropriate primers were designed containing both an ER and vacuolar targeting signals, (VTS). Those primers were used for PCR amplification of the human *GBA* gene (Hu-GBA) gene from constructed PGEM-GBA plasmid which was cloned in the plant expression vector pCAMBIA1304. The resulted construct was transported in *Agrobacterium tumefaciens* strain LBA4404 and was used for transformation of cotyledon explants. After 5-day of seedling, cotyledons were cut and used as explants. After infection and co-cultivation, hygromycin B was added in selection media as a selective agent for the transformants cotyledons. The presence of the Hu-GBA transgene in the genomes of transgenic plants was determined by polymerase chain reaction PCR as a band of size1587 bp. The GBA mRNA expression in modified soybean was detected by qRT-PCR compared with control GBA mRNA.

Keywords: signal peptide, Hu-GBA gene, pCAMBIA1304 vector, Soybean, *Agrobacterium*- mediated transformation

Introduction:

The concept of utilizing plants for the production of valuable pharmaceuticals, such as vaccines and recombinant proteins, was introduced over twenty years ago (1). Compared with bacteria, yeast, mammalian and insect cell culture expression systems, plants have many advantages for the production of "recombinant proteins" such as the very low production costs, safety, very high scale-up potential, and an initial decrease investment requirements (2). Molecular cultivation refers to the usage of plants and cell cultures to produce "recombinant proteins". Glycosylation is a major factor when attempting to produce recombinant proteins using *Escherichia coli* based expression system. Glycosylation is often requisite for many proteins to be folded properly, and can play an important role in protein stability and turnover rates (3). In additional non-native glycan structures can be immunogenic. However, recombinant proteins in plant will be modified when targeted for retention within the "endoplasmic reticulum (ER)" by the addition of high-mannose-type N-glycans (4). The immunogenic complex N-glycans and O-glycans are not added until later in the secretory pathway. Several studies have shown that recombinant proteins will be retained within the ER when they are modified by high-mannose-type N-glycans (5). Glucocerebrosidase, (GCD) is a "memberane-bound-lysosomal" enzyme that stimulates the analysis of glucocerebroside, (GlcCer) into "glucose and ceramide" (6). Gaucher disease is caused by the point mutations in the human *GBA gene* (*Hu-GBA*), which results in the aggregation of GlcCer in lysosomes of macrophages (7). The Hu-GBA, first reported in 1985 (8), consists of 497-amino-acids, which came from a 536-mer pro peptide. The mature human glucocerebrosidase (hGCD) has five

"N-glycosylation consensus sequences (Asn-X-Ser/Thr)". Glycosylation at the first site is requisite to produce the active protein (9). The native GCD is a glycoprotein containing four carbohydrates chains that does not aims phagocytic cells in the body and therefore has restricted therapeutic value (10). During the development of the present treatment for gaucher disease, the terminal sugars are sequentially removed from the carbohydrate chain of GCD (11) Three different glycosidases involved the formation of a glycoprotein with terminal mannose residues in order to target macrophages which bear mannose receptors (12).While many plant systems have been utilized for backing the expression of heterogeneous proteins, we thought that soybeans may be the most effective of these systems (13). Soybeans are often overlooked as a system of expression in part due to a difficult, lengthy and costly conversion process. However, there is enormous potential for using genetically modified soybeans as a plant to produce pharmaceutical proteins (14). The soybean system has distinctive features that make it a practical alternative to existing expression systems. First, although soybean seeds are traditionally considered highly oiled and proteinaceous seeds, protein represents 38% of the dry mass of soybeans, and is considered one of the richest known natural sources of protein. Offered this high protein content (15), transgenic proteins can be expressed at levels exceeding one milligram in a single soybean seed. There are few, plant systems that are capable of producing such high levels of foreign protein (16). Second, soybeans can be an easy to grow and relatively inexpensive plant. Therefore, the production of biopharmaceuticals in soybeans is very cost effective (17). The aim of this study is to confirm the success of the designed primers with plant signal peptide in amplified the *GBA gene* and the expression of this gene in soybean plant.

Materials and Methods:

Construction of expression cassette

The pGEM-GBA plasmid from Sino Biological Inc Cat.no.HG12038-G was used as the source for cDNA encoding human glucocerebrosidase (hGCD) (RefSeq: NM_000157), the pCAMBIA 1304 plant expression vector from Marker Gene Technologies, Inc., is controlled by the 35S promoter from cauliflower mosaic virus and terminated by the CaMV35S polyA signal. A gene-specific primer pair was designed according to the predicted sequence of GBA (NM_000157; NCBI Reference Sequence). GBA cDNA was then modified a designed forward primer containing DNA coding sequences for the ER targeting signal encoded by the basic endochitinase *gene* (*Arabidopsis thaliana*), ATGAAGACTAATCTTTTTCTCTTTCTCATCTTTTCACTTCTCCTATCATTATCCTCGGCCGAATTC which has been communicated to increase the accumulation of recombinant protein in plant tissues, and a reverse primer containing the vacuolar targeting signal GATCTTTTAGTCGATACTATG from tobacco chitinase A. Recognition sites for the *BglII* and *BstEII* restriction enzymes were inserted into the 5′and 3′ends of the designed primers , with the respective recognition site sequences shown in bold as following: The forward primer 5′**CTAGATCT**ATGAAGACTAATCTTTTTCTCTTTCTCATCTTTTCACTTCTCCTATCATTATCCTCGGCCGAATTCGCCCGCCCCTGCA 3′, and reverse primer 5′**AGCGGTCACC**GATCTTTTAGTCGATACTATGCTGGCGATGCCACAG3′.

Binary vector construction

The purified PCR product was sequenced and inserted into the binary vector pCAMBIA1304, after being digested with the endonucleases *BglII* and *BstEII*, yielding a pCAMBIA1304-GBA vector (Fig. 1). The ligation yield mixture was used to transform the *E. coli* strain DH5-α (Invitrogen) and kanamycin resistant colonies were segregated after overnight incubation at 37 °C. After amplification using the colony PCR method, the constructed plasmid was extracted from bacterial cells using AccuPrep® plasmid Mini Extraction Kit (Bioneer), and the plasmid confirmed by restriction enzyme digestion, PCR and sequencing. The plasmid was introduced into *A. tumefaciens* strain LBA4404 (Takara Bio Inc. Cat. # 9115) by the heat shock method. Transformed cells were screened for kanamycin-resistance and amplified by colony PCR.

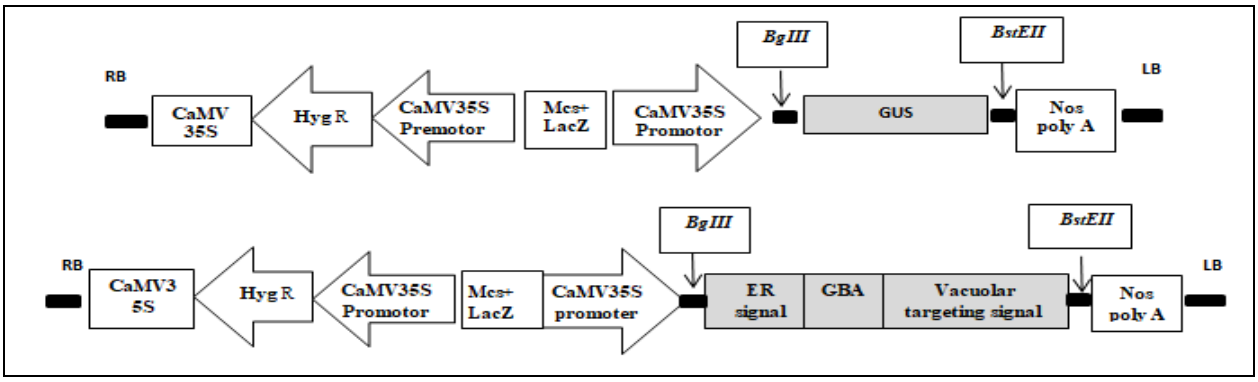

Figure 1. Schematic representation of the plant transformation vectors. (A) The *GUS* gene was excised out of binary vector pCAMBIA 1304 using the *BgIII* and *BstE*II restriction enzymes. (B) ER signal, *GBA* gene and Vacuolar targeting signal were inserted into the vector.

Plant materials and cultivation conditions

The *Agrobacterium* mediated transformation through mature Soybean [*Glycine max* L.] cotyledon was performed as previously described by (18) and (19) with some modifications.

Sterilization of soybean seeds and germination

Soybean [*Glycine max* L.] (Seeds were obtained from market in Egypt). Seed surfaces were sterilized using chlorine gas formed by mixing 3.5 mL 12 N HCl with 100 mL bleach (5.25% sodium hypochlorite) for 12 hrs. Sterilized seeds were germinated on basal MS germination medium supplement with B5, 2% sucrose and 0.7% agar (pH 5.8) The tissue culture jars were kept in an incubator at 25°C under 18/6 hrs. (light/dark) photoperiod conditions for 5-6 days.

Preparation of the *Agrobacterium* strain for infection

A single colony of *Agrobacterium* containing the pCAMBIA1304-GBA plasmid was cultured for 48 hrs. In yeast extract peptone (YEP) medium supplemented with 100 mg/L streptomycin and 50 mg/L kanamycin. After cells density reaching of OD_{600}=1.5, the culture was centrifuged at 5.000 r min−1 for 10 min , and the pellet was resuspended in infection medium (IM) (4.33 g /L MS medium supplemented with B5, 30 g/L sucrose, 3.3 mM L-cysteine, 1.68 mg/ L Benzylaminopurine (BAP), 0.25 mg/ L Gibberellic Acid (GA3),1Mm, sodium thiosulfate, 200mM acetosyringone (AS), 1mM dithiothreitol (DTT), and 200 mM 2-(N-morpholino) ethanesulfonic acid (MES), (pH 5.4). OD600 of 0.6 were applied for the infection step.

Preparation of explants and infection

When the cotyledons became green and the seed coat split open, they were separated and dipped into the previously prepared *Agrobacterium* suspension, and shaken at 50 rpm at 28 °C for 30 min. Then, the explants were blotted on sterile filter paper and placed on filter paper laid over the co-cultivation medium (CCM), CCM was composed of IM medium supplement with 0.6% agar Co-cultivation plates were incubated at 22 °C for 5 days, at 16 hrs. light/8 hrs. dark.

Selection, rooting and hardening

After co-cultivation, *Agrobacterium* was removed by washing the cotyledons three times with sterile water added with 50 mg/ L carbenicillin, on a shaker, at 410 rpm for 40 min (20). Explants were transferred to selective shoot induction medium (SSIM) composed of full strength MS medium supplemented with 30 g/L sucrose, 3 mM MES,1.68 mg/L BAP, 15 mg/L hygromycin, and 0.65% agar, (pH 5.4) (19) . After 2 weeks, the explants were moved to shoot elongation medium (SEM) full strength MS medium, supplemented with 30 g/L sucrose, 0.750 mg/L gibberellic acid, 3mM MES, glufosinate 5mg/L, 15 mg/L hygromycin and 0.65% agar, (pH 5.8) and cultured under18/6hrs. Light/ dark at 25℃. The explants were sub cultured on the same fresh medium at 14 days intervals. After the formed shoots reached approximately 2–3 cm in height, the regenerating shoots were planted in rooting induction media (RIM) half strength B5 medium was supplemented with 1% sucrose, 2 mg/L indole-3-butyric acid (IBA) and 0.7% agar, (pH 5.4) for 20-30 days. Plantlets were cultivated in rooting media until they developed roots.

Good rooted transformed soybean plants were hardening under controlled environment conditions by wrapping the pots with transparent bags for another weeks under18/6h light/dark at 25°C. The bags were then opened to acclimate for one week. The plantlets were irrigated once in 2days after which they were transferred to soil and vermiculite (1:1) and then grown in a greenhouse under the same climatic conditions.

Isolation of Hu-GBA

To identify *Hu-GBA* gene in transformed soybeans total RNA was reverse-transcribed into single-stranded cDNA, using the AccuPower®RocketScript™RT Premix (Bioneer, Korea). Using this cDNA as a template, specific primers (F5- CCATGGCTGGCTGGCATCACA-3 &R5 - CTGGCGATGCCACAG-3) were used to amplify *Hu-GBA*. The PCR reaction conditions were as follows: 94°C for 5 min, then 30 cycles at 94°C for 1 min, 58°C for 52 sec, and 68°C for 3 min, with a final extension at 68°C for 7 min. The sequences were aligned with the GBA sequence using BLAST (http://www.ncbi.nlm.nih.gov/BLAST).

Q R T- PCR assay

Qualitative real-time reverse transcription polymerase chain reaction (qRT- PCR) was performed to analyse *Hu-GBA* expression at the transcription level. The RNA was extracted from transformed plant leaf tissue using the GENEzol™TriRNA Pure Kit from Geneaid and oligo (dT) primer (Bioneer) to synthesise complementary DNA (cDNA) via reverse transcription, using the AccuPower® RocketScript™ RT Premix (Bioneer). The resulting cDNA mixture was used as a template for qRT-PCR. The expression of recombinant *GBA* was quantitative analysis using a qRT-PCR system (Bioneer). QRT-PCR was performed in a 20 µL reaction volume containing 1 µM of each primer and 10 µL AccuPower ® Green Star ™ qPCR PreMix Bioneer kit). The qRT-PCR experiment was performed using the forward primers 5-CAGCCTCACAGGTTTGCTTCT-3 and reverse primer was R-5GACACACACCGAGCTGTA-3, respectively. The expression of *Hu-GBA* in transformed plant was compared with the expression of Hu-GBA in the blood of healthy human as a positive control and with gene of untransformed soybean as negative control. The qRT-PCR instrument was programmed as follows: 1 min at 95 °C, 40 cycles of 35 sec at 95°C, 45 sec at 58°C and 1 min. at 68°C, followed by a final extension at 68 °C for 5 min.

Results

The original objective of this study was to modify the sequence of the natural human cDNA encoding for hGCD to permit its expression in soybean. The GCD signal peptide was substituted with that for the *Arabidopsis thaliana* basic endochitinase gene, and the storage vacuole targeting signal from tobacco chitinase A. The PCR products were analyzed using 1% agarose gel electrophoresis, and specific bands were observed at 1587bp, which included the 1,502 bp of an open reading frame of Hu-GBA, 64 bp targeting signal from basic endochitinase and 21 bp vacuolar targeting signal (Fig.2).

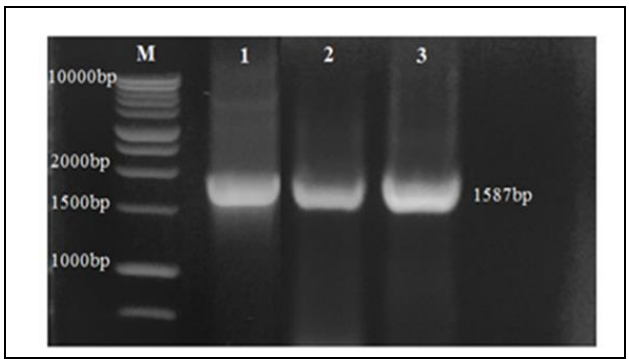

Figure 2. PCR analysis of 1587bp fragment of human *GBA1* gene amplification from pGEM-GBA plasmid. Lane 1-3, Lane M-1Kbp ladder.

Digestion of the recombinant plasmid with *BgIII* and *BstEII* restriction enzymes

After isolated pCAMBIA 1304-GBA, from transformed *E. coli DH5-α* was double digested with both enzymes *BgIII* and *BstEII*, resulted two separated bands (Fig. 3). The top band (9,826 bp) represented the DNA vector without the *GUS* gene and the bottom band represented the modified *GBA* with the ER and vacuolar targeting signals (approximately 1,587bp).

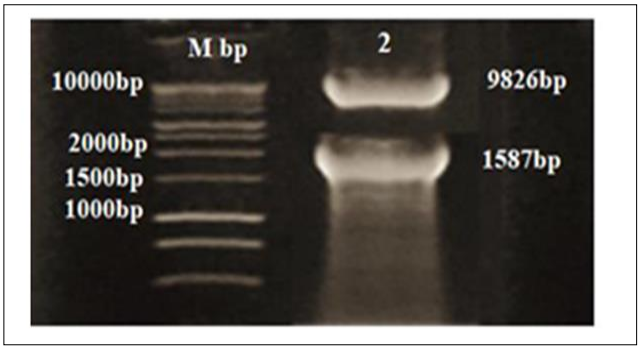

Figure 3. Restriction enzyme digestion analysis recombinant plasmide pCAMBIA1304-GBA, (Lane 2). Lane M1Kbp ladder

Transformation of pCAMBIA 1304-GBA into *Agrobacterium tumefaciens LBA4044*

The recombinant plasmid "pCAMBIA1304-GBA" was successfully converted into *A. tumefaciens* strain LBA4044, used calcium chloride transformed method and the transformed *A. tumefaciens* LBA4044 was selected on yeast extract peptone YEP medium with kanamycin and streptomycin antibiotics (Fig. 4). The colony PCR was used to scan GBA inserts in transformed bacteria. A 1,587bp fragment of was obtained using specific primers for *GBA gene* (Fig.5).

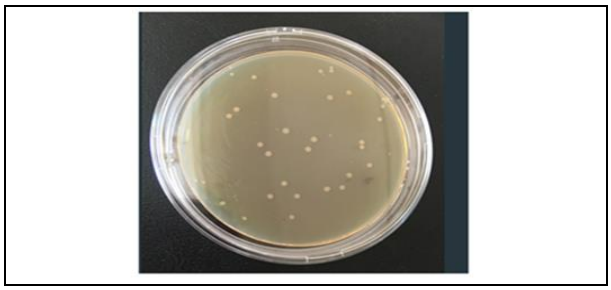

Figure 4. Transformed *A. tumefaciens* LBA4404 colonies with pCAMBIA1304 on LB plate contain streptomycin and kanamycin

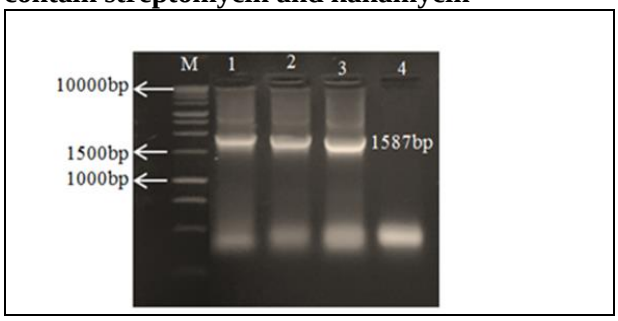

Figure 5. PCR amplified *GBA* from two different transformed *A. tumefaciens* colonies. Lane 1, 2: *GBA1* gene amplify from transformed bacteria. Lane 3: *GBA1* gene as positive control. Lane4: negative. M: 1 kbp DNA ladder

***Agrobacterium*-mediated transformation**

During the plant transformations, soybean, half-cotyledons were co-cultivated with *Agrobacterium* strain harboring the recombinant pCAMBIA1304-GBA plasmid for five days (Fig. 6C). Explants were placed onto the SIM for two weeks to promote the shoot formation (Fig. 6D). Two weeks later, shoots were transferred to SEM (Fig. 6E). After four to six weeks, the elongated shoots were transferred to RIM (Fig. 6F). Rooted plantlets were transplanted into small pots, containing soil mixture initially were covered with plastic dome and placed in an incubated (Fig.6g, h). The resulting T0 plantlets were then transplanted into bigger pots and grown in soil in a greenhouse (Fig. 6i)**.**

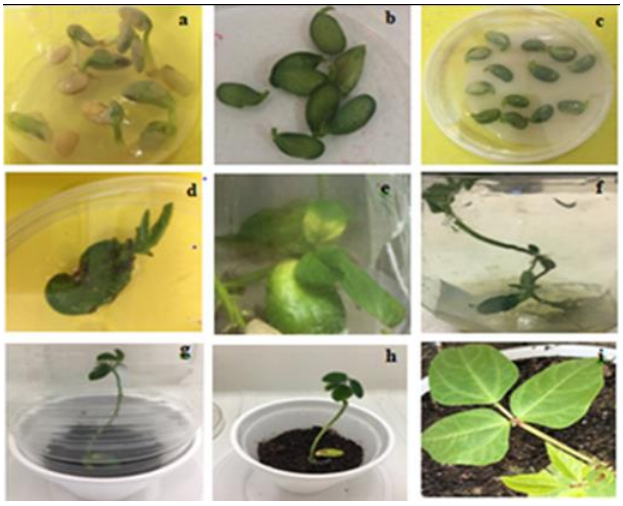

Figure 6.The experimental process of *Agrobacterium*-mediated transformation soybean explants (a) Sterile soybean seeds germinated on Germination media and after 5-day-old seedlings were harvested for the explants. (b) The pair of cotyledons was divided into two individual cotyledons, the attachment points of the cotyledon and hypocotyls were wounded with a sharp scapel. (c) After infection with *Agrobacterium*, the explants were dried on sterile filter paper and transferred to Co-cultivation covered with sterile filter paper, (d) Shoot induction. (e) Shoot elongation. (f) Rooting. (g, h,i) Transplanting and adaptation to normal growth condition.

Analysis of the transgenic plants

PCR analysis (Fig. 7) revealed the presence of the expected bands for Hu-GBA amplicons, at 1,587 bp which included the 1,502 bp Hu-GBA open reading frame, only in transgenic individuals. These PCR results were similar those for that of the positive control (Hu-GBA amplified from pGEM-GBA plasmid). Under the same conditions no similar band was detected in the untransformed control. The results confirmed the integration of the transgene into the plant genome. Further analysis of the putative transgenic plants, using quantitative RT-PCR, confirmed the successful transformation. These results in (Fig.8) demonstrated that *Hu-GBA* was successfully integrated into the soybean plant genome and was stably expressed in transgenic plants. The real time efficiency was calculated from the given slope in light cycle software. The ratio of the gene of interest to the positive control gene was calculated from threshold cycle (CT). GBA mRNA expression based on the transformed soybean was determined according to the CT method using a GBA mRNA expression in healthy human blood as positive control. The results showed that the expression of the *GBA* transcript in transformed

soybean was 1.868 compared with to the control value of 2.0.

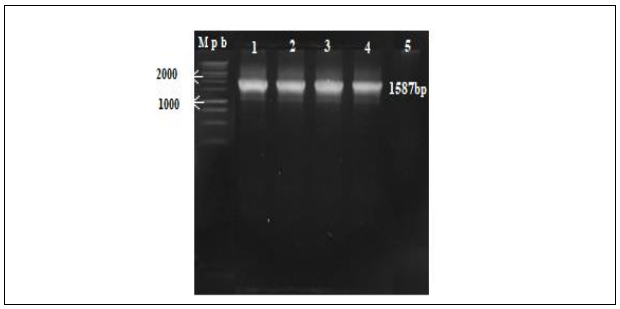

Figure7. PCR amplification of transgenic plant transformed with Pcambia1304-GBAconstract. Lane M:1 kb DNA ladder. Lane1: Positive control GBA gene. Lane2-4: GBA gene band amplified from transformed plant. Lane 5: Negative control from untransformed plant.

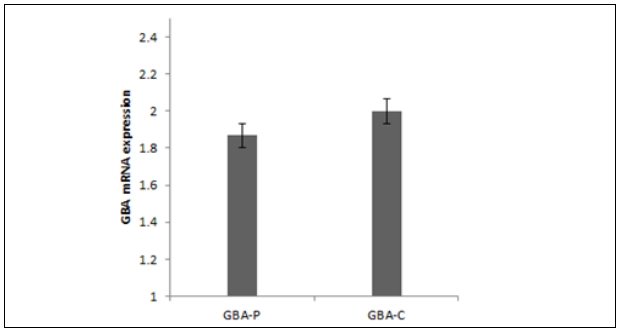

Figure8. Gene expressions of *GBA gene* in transformed soybean in compare with expression in human blood GBA-C mRNA form human Blood sample as positive control GBA-P mRNA of recombinant GBA gene from transformed soybean plants

Discussion:

In this study, the transformation of *Hu-GBA* in soybean was verified. Glycosylated proteins can be produced using a plant culture, particularly proteins with high levels of mannose glycosylation, and the proteins can be designed to target the ER and/or by-pass the Golgi using specific vectors resulting in the production of enzymatically active, high-mannose lysosomal enzymes using transgenic plants. In this study the nucleotide sequence of the *Hu-GBA* gene was modified, through PCR techniques, using designed primers, to fuse the signal peptide from *Arabidopsis thaliana* basic endochitinase and a C terminal vacuole targeting sequence from tobacco chitinaseA, to the *Hu-GBA* the sequence, as described by a previous study (20). Targeting the heterogeneous proteins to the appropriate organelles can be crucial to obtaining high levels of accumulation because the structure and stability of a recombinant protein are determined by its pathways and endpoints in the cell. The ER is the entry port for the secretory protein pathway (21), and the ER is the site where multiple newly synthesized peptides multiply and assemble and glycoproteins become glycosylated on aspartame residues (22). A 1,587bplong PCR product was cloned upstream from CaMV35S and downstream of the *NOS* terminator in the pCAMBIA1304 vector between *BstEII* and *BgIII* restriction sites (Fig.1). This construct was transformed into the *A. tumefaciens* strain LBA4404 after being isolated from transformed *E. coli* DH5α cells. The presence of *Hu-GBA* was confirmed by colony PCR (Fig. 3) and digestion reactions, which were conducted using the *BstEII* and *BglII* restriction enzymes (Fig.4). *Agrobacterium* infection plays a major role in the soybean transformation processes (23). Researchers have demonstrated that the cotyledonary node is good candidate explant for shoot regeneration (24). In *Agrobacterium*-mediated transformation systems, the cotyledonary node must first be wounded to release phenolic compounds and to provide access to the target cell. The wound enhances the activity of the internal cytokines to stimulate cell division (18). In this study, the cotyledons were cut because wounding is important for increasing the efficient plant transformation, as *Agrobacterium* can sense a wounded potential host by perceiving these phenolic compounds. The infection medium also contained AS and DTT and had an acidic pH, which are all factors that have been shown to facilitate T-DNA transfer and to enhance transformation efficiency (25). After 5 days of co-cultivation the moderate survival of explants was observed but with higher transformation efficiencies (26). In this study, DTT was added to the CCM as an antioxidant, to reduce pruning and necrosis in explants which represents one of the primary factors affecting the conversion efficiency (19). Cytokinins can activate cell division, and many reports have shown a direct link between cytokinins and cell cycle control (27). The transgenic soybeans were analysed by PCR and qRT-PCR, and these techniques demonstrated the steady expression of recombinant *GBA* in transgenic plants, at the RNA level. *GBA* could be amplified from genomic RNA isolated from transformed soybean leaves, using specific primers, as shown in (Fig.7), appeared to have the same band size as the control GBA which proved that the gene that amplified from transgenic plants was GBA. The q RT-PCR results (Fig. 8) showed that *GBA* was expressed in transformed soybeans plants, at relatively lower levels than in human blood. No GBA RNA was detected in non-transformed control plants. Quantitative real-time reverse transcription-polymerase chain reaction

(qRT–PCR) is the preferred method for confirming gene expression and detecting the fold change difference in mRNA levels for any target gene, due to its high sensitivity, specificity and reproducibility (28). The expression of *GBA* gene in transformed soybeans was confirmed when its RNA we converted to cDNA, and the *GBA* expression level was compared with than of blood sample from a healthy person. GBA mRNA in transgenic soybean plant was closed to its level in the human blood, and this may be an indication of the possibility of producing the glucocerebrosidase from soybeans and considering it as economic source for this enzyme.

Conclusions:

In this study, *Agrobacterium* successfully inserted Hu-GBA inside soybean genomic and generated transformed plants. In this protocol, the pCAMBIA 1304 plant expression vector was used as binary vector, soybean cotyledons were selected as explants. CDNA-GBA was modified using designed primers that contained both an ER targeting signal, and a vacuolar targeting signal. The qRT-PCR analysis showed that transgenic soybeans effectively expressed *Hu-GBA* with comparable levels as compared with those observed in healthy human blood.

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Author's declaration:

- Conflicts of Interest: None.
- I hereby confirm that all the Figures and Tables in the manuscript are mine. Besides, the Figures and images, which are not mine, have been given the permission for re-publication attached with the manuscript.
- Ethical Clearance: The project was approved by the local ethical committee in University of Basra.

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تصميم بادئات باشارات ببتيديه نباتية لتعزيز التعبيرالجيني لجين *GBA1* في نبات فول الصويا المحور

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الخلاصة:

تقدم النباتات المعدلة وراثيا مزايا لإنتاج بروتينات مركبة حاوية على جزيئات سكر المانوزفي طرف سلاسل الغليكان، لذلك يتم اختيار النباتات كمصدر للمنتجات الصيدلانيه بالاضافه الى تطوير انظمة تعبير بديله لانتاج إنزيمات الليزوزوميه المركبة. في الدراسة الحالية التتابع الجيني الطبيعي لجزيئة الديوكسي رايبوز ثلاثي الفوسفات المتممه والتي تشفر لانتاج الانزيم البشري الغلوكوسيربيروزيداز حورت لتعزيز التعبير الجيني لهذا الانزيم داخل نبات فول الصويا،حيث ان اشارة البدا لجين *GBA* تم استبدالها باشارة البدا لجين انزيم اندوجينيز لنبات *Arabidopsis thaliana* وذلك لتسهيل انتقال البروتين المركب الى الشبكه الاندوبلازميه كما تم اضافه التتابع الخاص باشاره جين الجيتينيز لنبات التبغ والمسؤل عن خزن البروتين المركب داخل حويصلات الخزن وذلك بتصميم بادئات خاصه لتضخيم الجين البشري *GBA* حاويه على كلا من اشاره النقل الى الشبكه الاندوبلازميه واشاره النقل الى الحويصلات الخازنه. استخدمت تقنية PCR لتضخيم Hu-GBA من البلازمد المركب PGEM-GBA بعدها تم كلونة الجين المضخم في ناقل التعبير النباتي المسمى pCAMBIA1304 داخل *Agrobacterium tumefaciens* سلالة LBA4404 والتي بدورها استخدمت لتحويل فلقات نبات فول الصويا، حيث أن الفلقه وبعمر 5ايام قطعت واستخدمت كنموذج للاستزراع وبعد اصابة الفلقات بالبكتريا المحوله واعادة استنباتها استخدم مضاد الهايكرومايسين بي في وسط الاختبار لاختيار الفلقات المحورة وراثيا. استخدمت تقنيةPCR لتحديد وجود *GBA*في جينومك النبات المحور وكانت نتيجة التضخيم حزمه بحجم 1587زوج قاعدي، كما تم الكشف عن التعبير الجيني للجين في نباتات فول الصويا المحور بواسطة الريال تايم مقارنة مع Hu-GBA القياسي

الكلمات المفتاحية: إشارة الببتيد، جين Hu-GBA، ناقل pCAMBIA1304، فول الصويا، الجراثيم، التحول بوساطة Agrobacterium-