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(54) Benævnelse: **ALPHA-GALACTOSIDASE-SAMMENSÆTNINGER**

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DESCRIPTION

FIELD AND BACKGROUND OF THE INVENTION

[0001] The present invention, in some embodiments thereof, relates to a nucleic acid expression construct and, more particularly, but not exclusively, to a nucleic acid construct for expression of α -galactosidase in plant cells, cells expressing the nucleic acid construct and the human α -galactosidase, and uses thereof.

[0002] Fabry disease is an X-linked lysosomal storage disease that is caused by deficient activity of lysosomal enzyme α -galactosidase A (α -Gal A). Patients with classic Fabry disease typically have α -Gal A activity of less than 1% and often demonstrate the full spectrum of symptoms, including severe pain in the extremities (acroparesthesias), hypohidrosis, corneal and lenticular changes, skin lesions (angiokeratoma), renal failure, cardiovascular disease, pulmonary failure, neurological symptoms and stroke. In atypical Fabry disease, individuals with residual enzyme activity demonstrate symptoms later in life, and the symptoms are usually limited to one or a few organs. Clinical manifestations in female carriers vary greatly because of random X-chromosome inactivation. Although carriers commonly remain asymptomatic throughout life, many demonstrate clinical symptoms as variable and severe as those of affected males. De Duve first suggested that replacement of the missing lysosomal enzyme with exogenous biologically active enzyme might be a viable approach to treatment of lysosomal storage diseases [Fed Proc. 23:1045 (1964)].

[0003] Recombinant α -Galactosidase A for enzyme replacement therapy has been produced in insect (sf9) cells (see US 7,011,831) in human fibroblasts (see US 6,395,884) and in plant cells (see US 6,846,968 and WO2008/132743). Clinical trials with recombinant α -Gal A (agalsidase beta [Fabrazyme®]: Genzyme Corporation, Cambridge, Mass; agalsidase alfa [Replagal®]: Shire Human Genetic Therapies Corporation, Cambridge, Mass) have been performed, and both drugs have been approved for clinical use. As of 2009, more than 2000 patients had been treated with either one of the two available formulations, and in general, safety and efficacy of both have been demonstrated (Hoffmann, Orphanet J Rare Dis. 2009;4:21).

[0004] Clinical experience has indicated that the available recombinant enzyme compositions differ with regard to patient tolerance, immunogenicity, dosage regimen and clinical efficacy (Hoffmann, Orphanet J Rare Dis. 2009;4:21). Another drawback associated with the existing recombinant enzymes is their expense, which can place a heavy economic burden on health care systems. The high cost of these recombinant enzymes, when produced in mammalian cell cultures, bioreactors or insect cells results from a complex purification and modification protocol, and the relatively large amounts of the therapeutic required for existing treatments. There is therefore, an urgent need to reduce the cost of α -galactosidase so that this life saving therapy can be provided more affordably to all who require it.

[0005] Human lysosomal enzymes can be produced in transgenic plants in order to solve problems of safety, viral infections, immune reactions, production yield and cost. US 2002/0088024 teaches expression in plant cells of recombinant human α -galactosidase, using a rice-amylase ER targeting signal peptide, and a human α -galactosidase coding sequence truncated at the C-terminal portion for efficient expression and secretion into the intracellular fluid.

[0006] WO 97/10353 teaches production of lysosomal enzymes in plants, specifically IDUA and glucocerebrosidase, using the wound-induced MeGA promoter and FLAG tag for recovery. WO 97/10353 does not provide guidance for specific constructs expressing recombinant human α -galactosidase.

[0007] Cramer et al. (Curr. Topics Trans. 1999 Plants 95-118) reviews methods of expression of glucocerebrosidase and IDUA in plants in a similar manner as WO 97/10353, that is in the endomembrane (IF) of tobacco leaves, and describes plant expression in general but does not provide guidance for expression of human recombinant α -galactosidase.

[0008] US2006-0204487 teaches methods for the expression of human lysosomal enzymes in plants, employing a variety of strategies for targeting the recombinant polypeptides (specifically, glucocerebrosidase) for efficient glycosylation and glycan remodeling. Cloning and expression of recombinant human α -galactosidase from specific constructs is not disclosed.

[0009] WO2007/010533 teaches the administration of plant cells expressing recombinant bioactive molecules, for example, lysosomal enzymes such as human glucocerebrosidase and human α -galactosidase. Cloning and expression of recombinant human α -galactosidase is not disclosed.

[0010] WO2008/132743 describes cloning of a human α -galactosidase coding sequence into a plant expression vector, and its expression in tobacco cells, resulting in a biologically active, full length glycosylated α -galactosidase enzyme which demonstrated uptake in fibroblasts. However, more advanced α -galactosidase expression vectors for yet further increased efficiency of expression and production of recombinant human α -galactosidase protein having improved pharmacokinetics under clinical conditions are needed.

SUMMARY OF THE INVENTION

[0011] The invention is as defined in the claims. According to an aspect of some embodiments of the present invention there is provided a nucleic acid expression construct, the construct comprising a nucleic acid sequence encoding a human α -galactosidase protein, wherein the human α -galactosidase protein is translationally fused at the N-terminal to an *Arabidopsis* ABPI endoplasmic reticulum targeting signal peptide and wherein the human α -galactosidase protein is translationally fused at the C-terminal to an endoplasmic reticulum retention signal peptide.

[0012] According to an aspect of some embodiments of the present invention there is provided a human α -galactosidase protein having an N-terminal Glycine residue, wherein the human α -galactosidase protein is translationally fused at the C-terminal to an endoplasmic reticulum retention signal peptide.

[0013] According to some embodiments of the invention, the nucleic acid sequence comprises SEQ ID NO: 22.

[0014] According to some embodiments of the invention, the *Arabidopsis ABPI* endoplasmic reticulum targeting signal peptide is as set forth in SEQ ID NO: 4.

[0015] According to some embodiments of the invention, the nucleic acid construct comprises a nucleic acid sequence encoding the endoplasmic reticulum targeting signal peptide as set forth in SEQ ID NO: 5.

[0016] According to some embodiments of the invention, the endoplasmic reticulum retention signal peptide is as set forth in SEQ ID NO: 6.

[0017] According to some embodiments of the invention, the nucleic acid construct comprises a nucleic acid sequence encoding the endoplasmic reticulum retention peptide as set forth in SEQ ID NO: 19.

[0018] According to some embodiments of the invention, the nucleic acid sequence has a sequence as set forth in SEQ ID NO: 2

[0019] According to some embodiments of the invention, the human α -galactosidase protein has an amino acid sequence as set forth in SEQ ID NO: 1.

[0020] According to some embodiments of the invention, a codon usage of the nucleic acid sequence is optimized for *Nicotinia tabaccum*.

[0021] According to some aspects of some embodiments of the invention there is provided an isolated cell comprising the nucleic acid construct of the invention.

[0022] According to some embodiments of the invention, the cell recombinantly produces the human α -galactosidase enzyme.

[0023] According to some embodiments of the invention, the human α -galactosidase comprises an N-terminal Glycine residue.

[0024] The cell is a plant cell.

[0025] According to some embodiments of the invention, the plant cell is a tobacco cell.

[0026] According to some embodiments of the invention, the tobacco cell is a BY-2 cell.

[0027] According to some embodiments of the invention, the human α -galactosidase protein is recombinantly produced so as to have at least one exposed mannose residue.

[0028] According to some embodiments of the invention, the human α -galactosidase protein is recombinantly produced so as to have at least one core β -(1,2) xylose or at least one core α -(1,3) fucose or both.

[0029] According to some embodiments of the invention, the nucleic acid construct is stably integrated into the genome of the cell.

[0030] According to some embodiments of the invention, the cell is an *Agrobacterium tumefaciens* cell.

[0031] According to an aspect of some embodiments of the present invention there is provided a method of producing a recombinant human α -galactosidase protein, comprising: providing a cell according to the invention; and growing the cell so as to produce the recombinant human α -galactosidase protein; and isolating the recombinant human α -galactosidase protein from the cell.

[0032] According to some embodiments of the invention, the cell is an isolated cell cultured in a cell culture medium.

[0033] According to some embodiments of the invention, the culturing is affected in a disposable bioreactor.

[0034] According to some embodiments of the invention, the human α -galactosidase protein, has an amino acid sequence as set forth in SEQ ID NO: 16.

[0035] According to an aspect of some embodiments of the present invention there is provided a pharmaceutical composition comprising, as an active ingredient, the human α -galactosidase protein of the present invention and a pharmaceutically acceptable carrier.

[0036] According to an aspect of some embodiments of the present invention there is provided a pharmaceutical composition comprising, as an active ingredient, the cell of the present invention and a pharmaceutically acceptable carrier.

[0037] According to an aspect of some embodiments of the present invention there is provided a pharmaceutical composition comprising, as an active ingredient, a population of human α -galactosidase proteins of the invention, wherein the predominant glycan structures of said population of human α -galactosidase proteins are mannose 4- β -(1,2) xylose (M4X); mannose 3- β -(1,2) xylose- α -(1,3) fucose [Fc(3)M3X] and mannose 8 (M8), and a pharmaceutically acceptable carrier.

[0038] According to an aspect of some embodiments of the present invention there is provided a method of treating Fabry's disease in subject in need thereof, comprising administering to the subject the pharmaceutical composition of the invention.

[0039] According to an aspect of some embodiments of the present invention there is provided a pharmaceutical composition comprising the human α -galactosidase protein of the invention, having a glycan structure comprising nine mannose residues, wherein three are exposed mannose residues, and a pharmaceutically acceptable carrier. The human α -galactosidase protein having a glycan structure comprising nine mannose residues, wherein three are exposed mannose residues can be 0.5%, 0.8%, 1.0%, 1.3%, 2% or more of the population of human α -galactosidase proteins of the composition.

[0040] According to an aspect of some embodiments of the present invention there is provided a method of treating Fabry's disease in subject in need thereof, comprising administering to the subject the pharmaceutical composition comprising the cell of the invention.

[0041] According to an aspect of some embodiments of the present invention there is provided the use of the human α -galactosidase protein of the invention for the manufacture of a medicament for the treatment of Fabry's disease in a subject in need thereof.

[0042] Unless otherwise defined, all technical and/or scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which the invention pertains. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of embodiments of the invention, exemplary methods and/or materials are described below. In case of conflict, the patent specification, including definitions, will control. In addition, the materials, methods, and examples are illustrative only and are not intended to be necessarily limiting.

BRIEF DESCRIPTION OF THE DRAWINGS

[0043] Some embodiments of the invention are herein described, by way of example only, with reference to the accompanying drawings. With specific reference now to the drawings in detail, it is stressed that the particulars shown are by way of example and for purposes of illustrative discussion of embodiments of the invention. In this regard, the description taken with the drawings makes apparent to those skilled in the art how embodiments of the invention may be practiced.

[0044] In the drawings:

FIG. 1 is an amino acid sequence alignment of the α -galactosidase encoded by the nucleic acid of the invention (SEQ ID NO: 1, lower sequence), and native human α -galactosidase protein (GenBank: X05790, upper sequence), including the native signal leader peptide (SEQ

ID NO: 3, highlighted in red). The *Arabidopsis* ABPI endoplasmic reticulum targeting signal peptide (SEQ ID NO: 4) is highlighted in green. The SEKDEL endoplasmic reticulum retention signal peptide (SEQ ID NO: 6) is located at the C-terminus of the plant-expressed protein. The mature native α -galactosidase enzyme protein sequence (SEQ ID NO: 7) is highlighted in yellow.

FIG. 2 is a scan of an SDS-PAGE gel illustrating expression levels of diverse human α -galactosidase constructs in plants. Extracts of leaves from *N. benthamain*a plants infiltrated with different human α -galactosidase constructs were separated on a 12% SDS-PAGE reduced gel, and stained with Coomassie Blue for visualization of protein. Lane 1= products of expression of a construct (SEQ ID NO:8) encoding an α -galactosidase with an apple pectinase endoplasmic reticulum targeting signal peptide (apple pectinase signal peptide=SEQ ID NO: 9). Lane 2= products of expression of SEQ ID NO: 2, encoding an α -galactosidase with an ABPI endoplasmic reticulum targeting signal peptide (ABPI signal peptide = SEQ ID NO: 4). Lane 3= products of expression of SEQ ID NO: 11, encoding SEQ ID NO:12 with an apple pectinase endoplasmic reticulum targeting signal peptide (SEQ ID NO: 9). Lane 4= Negative (no α -galactosidase coding sequence) control. Prominent band in lane 2 (arrow) corresponds to size of the mature recombinant α -galactosidase monomer (SEQ ID NO: 21). Coomassie stained band indicates abundant α -galactosidase protein;

FIG. 3 is a histogram demonstrating levels of catalytic activity in extracts of plants expressing diverse human α -galactosidase constructs. Leaves from *N. benthamain*a plants were infiltrated with different human α -galactosidase constructs as follows: A= SEQ ID NO: 8, encoding an α -galactosidase protein with an apple pectinase endoplasmic reticulum targeting signal peptide (apple pectinase signal peptide = SEQ ID NO: 9). B= SEQ ID NO: 2, encoding an α -galactosidase protein with an ABPI endoplasmic reticulum targeting signal peptide (ABPI signal peptide = SEQ ID NO: 4). C= SEQ ID NO: 13, encoding an α -galactosidase protein with an endochitinase B endoplasmic reticulum targeting signal peptide (endochitinase signal protein =SEQ ID NO: 14). D= SEQ ID NO: 11, encoding an α -galactosidase protein (SEQ ID NO: 12) with an apple pectinase endoplasmic reticulum targeting signal peptide (apple pectinase signal sequence = SEQ ID NO: 9). Leaves from the respective plants were extracted in activity assay buffer, and catalytic activity determined using *p*-nitrophenylalpha-D-galactopyranoside (*p*NP-alpha-D-Gal, GBB1290, IRIS Biotech, Germany) as a hydrolysis substrate. Generation of the *p*-nitrophenolate chromophore was monitored at 405 nm;

FIG. 4 is a graph comparing stability of two plant recombinant human α -galactosidases in human plasma (37 °C) measured by residual catalytic activity. α -galactosidase from leaves of *N. benthamain*a plants transformed with SEQ ID NO: 11, encoding SEQ ID NO:12 with an apple pectinase endoplasmic reticulum targeting signal peptide (open squares) and α -galactosidase from leaves of *N. benthamain*a plants transformed with SEQ ID NO: 2, encoding an α -galactosidase (SEQ ID NO: 1) having an ABPI endoplasmic reticulum targeting signal peptide (closed squares) was purified by plant disruption, salting out and chromatography, and incubated in human plasma at 37 °C for up to 1 hour, to simulate *in-vivo* pharmacokinetics. Samples were assayed for α -galactosidase catalytic activity by the PNP-G assay at indicated intervals. Note the greater stability of α -galactosidase from leaves of *N. benthamain*a plants

expressing SEQ ID NO: 1 (closed squares);

FIG. 5 is a graph comparing stability of catalytic activity of the plant cell expressed α -galactosidase (closed squares) with that of commercial human recombinant α -galactosidase expressed in human cell culture (Replagal®, open triangles). Unpurified extract of BY2 cells expressing SEQ ID NO: 2, encoding an α -galactosidase (SEQ ID NO: 1) having an ABPI endoplasmic reticulum targeting signal peptide (closed squares) and human recombinant α -galactosidase expressed in mammalian cell culture (Replagal®, open triangles), were incubated in plasma at 37 °C for up to 1 hour, to simulate *in-vivo* pharmacokinetics. Samples were assayed for α -galactosidase catalytic activity by the PNP-G assay at indicated intervals. Note the similar stability of the plant-cell-expressed and human-cell-expressed enzymes;

FIG. 6 is a graph comparing stability of catalytic activity of the plant expressed α -galactosidase (closed squares) with that of commercial human recombinant α -galactosidase expressed in human cell culture (Replagal®, open triangles). Unpurified extract of BY2 cells expressing SEQ ID NO: 2, encoding an α -galactosidase (SEQ ID NO: 1) having an ABPI endoplasmic reticulum targeting signal peptide (closed squares) and human recombinant α -galactosidase expressed in mammalian cell culture (Replagal®, open triangles), were incubated under conditions simulating the lysosomal environment [acidic (pH 4.6)] at 37 °C for up to 11 days. Samples were assayed for α -galactosidase catalytic activity by the PNP-G assay at indicated intervals, when incubated. Note the similar stability of the plant-expressed and mammalian-expressed enzymes for up to 9 days;

FIG. 7 is chromogram showing the distribution of glycan structures in the products of glycosylase digests of plant cell expressed α -galactosidase. Samples of extract of BY2 cells expressing SEQ ID NO: 2, encoding an α -galactosidase (SEQ ID NO: 1) having an ABPI endoplasmic reticulum targeting signal peptide were reduced, alkylated and separated on SDS-PAGE. The protein bands were taken for glycan analysis by trypsin digestion followed by both PNGase A and PNGase F digestion. The resulting free glycans were extracted, purified, labeled with the fluorescent reagent anthranilamide (2AB), separated using a TSK gel Amide 80 normal phase HPLC, and detected using a fluorescence detector. Note major peaks at 5.9 and 8.9 GU (84.55 minutes and 108.05 minutes), and a minor peak of M9 at 112.35;

FIG. 8 is a table showing the glycan profile the plant cell expressed α -galactosidase, expressed as the percent area of the individual glycans represented in the chromogram of Figure 7. Note the identical profiles of each independent analysis (A and B).

DESCRIPTION OF SPECIFIC EMBODIMENTS OF THE INVENTION

[0045] The present invention, in some embodiments thereof, relates to an expression vector for recombinant expression of human α -galactosidase in plant cells, methods for its expression thereof in plant cells and the human α -galactosidase produced thereby.

[0046] It is to be understood that the invention is not necessarily limited in its application to the details set forth in the following description or exemplified by the Examples. The invention is capable of other embodiments or of being practiced or carried out in various ways.

[0047] The present inventors have constructed an expression vector for recombinant expression of human α -galactosidase in plant cells, transformed tobacco plants and plant cells with the vector, and have isolated catalytically active human α -galactosidase from the plants and cell cultures. The expressed recombinant human α -galactosidase retained favorable catalytic activity under conditions simulating those normally encountered in *in-vivo* administration, suggesting improved simulated pharmacokinetics as compared to other plant derived recombinant human α -galactosidase, and similar to those of mammalian-expressed recombinant human α -galactosidase (Replagal®).

[0048] Thus, according to one aspect of the present invention, there is provided a nucleic acid expression construct, the construct comprising a nucleic acid sequence encoding a human α -galactosidase protein, wherein the human α -galactosidase protein is translationally fused at the N-terminal to an *Arabidopsis* ABPI endoplasmic reticulum targeting signal peptide and wherein the human α -galactosidase protein is translationally fused at the C-terminal to an endoplasmic reticulum retention signal peptide.

[0049] As used herein the term "human α -galactosidase protein" refers to a human α -galactosidase A (a-D-galactoside galactohydrolase; EC 3.2.1.22; α -Gal A) polypeptide. According to some embodiments of the invention, the human α -galactosidase protein is the mature human α -galactosidase protein, having the amino acid sequence as set forth in SEQ ID NO: 7 (mature human A-Gal). It will be appreciated that the human α -galactosidase protein can be a modified human α -galactosidase protein, having an amino acid sequence different than that of SEQ ID NO: 7. One non-limiting example of a modified human α -galactosidase protein encoded by the expression vector of the invention is SEQ ID NO: 16 (G-A-Gal). In another non-limiting example the human α -galactosidase protein encoded by the expression vector can be a mixture of human α -galactosidase proteins comprising both α -galactosidase proteins having an N-terminal glycine residue (G), such as SEQ ID NO: 16 or SEQ ID NO: 21, and absent an N-terminal glycine residue, such as, but not limited to SEQ ID NO: 7 and SEQ ID NO: 20.

[0050] According to another aspect of the invention, the human α -galactosidase protein is translationally fused at the N-terminal to an *Arabidopsis* ABPI endoplasmic reticulum targeting signal peptide and the human α -galactosidase protein is also translationally fused at the C-terminal to an endoplasmic reticulum retention signal peptide.

[0051] As used herein the term "translationally fused at the N-terminal" or "translationally fused at the C-terminal" refers to covalent attachment of the indicated peptide via a peptide bond to the N-terminal or C-terminal amino acid of the mature human α -galactosidase protein typically as a result of recombinant expression.

[0052] As used herein, the term "Arabidopsis ABPI endoplasmic reticulum targeting signal

"peptide" refers to the leader peptide sequence of the *Arabidopsis thaliana* auxin binding protein (Uni-Prot Accession No. P33487), which is capable of directing the expressed protein to the endoplasmic reticulum within the plant cell. In one embodiment, the *Arabidopsis* ABPI endoplasmic reticulum targeting signal peptide is a 33 amino acid polypeptide as set forth in SEQ ID NO: 4.

[0053] As used herein, the term "endoplasmic reticulum retention signal peptide" refers to a peptide sequence which, when present at the N- or C- terminus of a polypeptide, causes the polypeptide to be retrieved from the Golgi apparatus, and retained in the endoplasmic reticulum (see Rayon et al. *Journal of Experimental Botany*, Vol. 49, No. 326, pp. 1463-1472, 1998; and Neumann, et al *Annals of Botany*, 2003;92:167-180). In one embodiment, the endoplasmic reticulum retention signal peptide is KDEL (SEQ ID NO: 17) or SEKDEL (SEQ ID NO: 6). Other suitable plant endoplasmic reticulum signals are known in the art (see Pagny et al, *Journal of Experimental Botany*, Vol. 50, pp. 157-64).

[0054] Thus, according to an embodiment of the present invention, the human α -galactosidase protein translationally fused at the N-terminal to an *Arabidopsis* ABPI endoplasmic reticulum targeting signal peptide and the human α -galactosidase protein and translationally fused at the C-terminal to an endoplasmic reticulum retention signal peptide has an amino acid sequence as set forth in SEQ ID NO: 1.

[0055] As used herein the term "nucleic acid sequence" refers to a single or double stranded nucleic acid sequence which is isolated and provided in the form of an RNA sequence, a complementary polynucleotide sequence (cDNA), a genomic polynucleotide sequence and/or a composite polynucleotide sequences (e.g., a combination of the above).

[0056] As used herein the phrase "complementary polynucleotide sequence" refers to a sequence, which results from reverse transcription of messenger RNA using a reverse transcriptase or any other RNA dependent DNA polymerase. Such a sequence can be subsequently amplified *in vivo* or *in vitro* using a DNA dependent DNA polymerase.

[0057] As used herein the phrase "genomic polynucleotide sequence" refers to a sequence derived (isolated) from a chromosome and thus it represents a contiguous portion of a chromosome.

[0058] As used herein the phrase "composite polynucleotide sequence" refers to a sequence, which is at least partially complementary and at least partially genomic. A composite sequence can include some exon sequences required to encode the polypeptide of the present invention, as well as some intronic sequences interposing therebetween. The intronic sequences can be of any source, including of other genes, and typically will include conserved splicing signal sequences. Such intronic sequences may further include cis acting expression regulatory elements.

[0059] According to some embodiments of the present invention, the nucleic acid sequences

encoding the polypeptides of the present invention are optimized for expression. Examples of such sequence modifications include, but are not limited to, an altered G/C content to more closely approach that typically found in the plant species of interest, and the removal of codons atypically found in the plant species commonly referred to as codon optimization. In one embodiment, the codon usage of the nucleic acid sequence encoding the human α -galactosidase protein translationally fused at the N-terminal to an Arabidopsis ABPI endoplasmic reticulum targeting signal peptide and the human α -galactosidase protein and translationally fused at the C-terminal to an endoplasmic reticulum retention signal peptide is optimized for *Nicotiana tabacum* or *Nicotiana benthamiana*.

[0060] The phrase "codon optimization" refers to the selection of appropriate DNA nucleotides for use within a structural gene or fragment thereof that approaches codon usage within the plant of interest. Therefore, an optimized gene or nucleic acid sequence refers to a gene in which the nucleotide sequence of a native or naturally occurring gene has been modified in order to utilize statistically-preferred or statistically-favored codons within the plant. The nucleotide sequence typically is examined at the DNA level and the coding region optimized for expression in the plant species determined using any suitable procedure, for example as described in Sardana et al. (1996, Plant Cell Reports 15:677-681). In this method, the standard deviation of codon usage, a measure of codon usage bias, may be calculated by first finding the squared proportional deviation of usage of each codon of the native gene relative to that of highly expressed plant genes, followed by a calculation of the average squared deviation. The formula used is: $1 \text{ SDCU} = n = 1 N [(X_n - Y_n) / Y_n]^2 / N$, where X_n refers to the frequency of usage of codon n in highly expressed plant genes, where Y_n to the frequency of usage of codon n in the gene of interest and N refers to the total number of codons in the gene of interest. A Table of codon usage from highly expressed genes of dicotyledonous plants is compiled using the data of Murray et al. (1989, Nuc Acids Res. 17:477-498).

[0061] One method of optimizing the nucleic acid sequence in accordance with the preferred codon usage for a particular plant cell type is based on the direct use, without performing any extra statistical calculations, of codon optimization Tables such as those provided on-line at the Codon Usage Database through the NIAS (National Institute of Agrobiological Sciences) DNA bank in Japan ([Hypertext Transfer Protocol://World Wide Web \(dot\) kazusa \(dot\) or \(dot\) jp/codon/](http://Hypertext Transfer Protocol://World Wide Web (dot) kazusa (dot) or (dot) jp/codon/)). The Codon Usage Database contains codon usage tables for a number of different species, with each codon usage Table having been statistically determined based on the data present in Genbank.

[0062] By using the above Tables to determine the most preferred or most favored codons for each amino acid in a particular species (for example, rice), a naturally-occurring nucleotide sequence encoding a protein of interest can be codon optimized for that particular plant species. This is effected by replacing codons that may have a low statistical incidence in the particular species genome with corresponding codons, in regard to an amino acid, that are statistically more favored. However, one or more less-favored codons may be selected to delete existing restriction sites, to create new ones at potentially useful junctions (5' and 3' ends to add signal peptide or termination cassettes, internal sites that might be used to cut and

splice segments together to produce a correct full-length sequence), or to eliminate nucleotide sequences that may negatively effect mRNA stability or expression.

[0063] The desired encoding nucleotide sequence may already, in advance of any modification, contain a number of codons that correspond to a statistically-favored codon in a particular plant species. Therefore, codon optimization of the native nucleotide sequence may comprise determining which codons, within the desired nucleotide sequence, are not statistically-favored with regards to a particular plant, and modifying these codons in accordance with a codon usage table of the particular plant to produce a codon optimized derivative. A modified nucleotide sequence may be fully or partially optimized for plant codon usage provided that the protein encoded by the modified nucleotide sequence is produced at a level higher than the protein encoded by the corresponding naturally occurring or native gene. Construction of synthetic genes by altering the codon usage is described in for example PCT Patent Application 93/07278.

[0064] According to some embodiments of the invention, the human α -galactosidase protein is encoded by a nucleic acid sequence as set forth in SEQ ID NO: 18. According to further embodiments of the invention, the *Arabidopsis* ABPI endoplasmic reticulum targeting signal peptide is encoded by a nucleic acid sequence as set forth in SEQ ID NO: 5. According to yet further embodiments of the invention the endoplasmic reticulum retention signal peptide is encoded by a nucleic acid sequence as set forth in SEQ ID NO: 19. According to still further embodiments of the invention the human α -galactosidase protein translationally fused at the N-terminal to an *Arabidopsis* ABPI endoplasmic reticulum targeting signal peptide and the human α -galactosidase protein and translationally fused at the C-terminal to an endoplasmic reticulum retention signal peptide is encoded by a nucleic acid sequence as set forth in SEQ ID NO: 2.

[0065] As used herein the term "nucleic acid expression construct" refers to a nucleic acid construct which includes the nucleic acid of some embodiments of the invention (e.g., SEQ ID NO: 2) and at least one promoter for directing transcription of nucleic acid in a host plant cell. Further details of suitable transformation approaches are provided hereinbelow.

[0066] According to some embodiments of the invention, there is provided a nucleic acid expression construct comprising the nucleic acid sequence of the invention, and a promoter for directing transcription of the nucleic acid sequence in a plant host cell.

[0067] According to some embodiments of the invention, the nucleic acid is operably linked to the promoter sequence.

[0068] A coding nucleic acid sequence is "operably linked" to a regulatory sequence (e.g., promoter) if the regulatory sequence is capable of exerting a regulatory effect on the coding sequence linked thereto.

[0069] Any suitable promoter sequence can be used by the nucleic acid construct of the present invention. Preferably the promoter is a constitutive promoter, a tissue-specific, or an

inducible promoter.

[0070] As used herein the phrase "plant-expressible" refers to a promoter sequence, including any additional regulatory elements added thereto or contained therein, is at least capable of inducing, conferring, activating or enhancing expression in a plant cell, tissue or organ, preferably a monocotyledonous or dicotyledonous plant cell, tissue, or organ. Such a promoter can be constitutive, i.e., capable of directing high level of gene expression in a plurality of tissues, tissue specific, i.e., capable of directing gene expression in a particular tissue or tissues, inducible, i.e., capable of directing gene expression under a stimulus, or chimeric, i.e., formed of portions of at least two different promoters.

[0071] Examples of preferred promoters useful for the methods of some embodiments of the invention are presented in Table I, II, III and IV.

Table I

<i>Exemplary constitutive promoters for use in the performance of some embodiments of the invention</i>		
<i>Gene Source</i>	<i>Expression Pattern</i>	<i>Reference</i>
Actin	constitutive	McElroy et al, Plant Cell, 2: 163-171, 1990
CAMV 35S	constitutive	Odell et al, Nature, 313: 810-812, 1985
CaMV 19S	constitutive	Nilsson et al., Physiol. Plant 100:456-462, 1997
GOS2	constitutive	de Pater et al, Plant J Nov;2(6):837-44, 1992
ubiquitin	constitutive	Christensen et al, Plant Mol. Biol. 18: 675-689, 1992
Rice cyclophilin	constitutive	Bucholz et al, Plant Mol Biol. 25(5):837-43, 1994
Maize H3 histone	constitutive	Lepetit et al, Mol. Gen. Genet. 231: 276-285, 1992
Actin 2	constitutive	An et al, Plant J. 10(1):107-121, 1996

Table II

<i>Exemplary seed-preferred promoters for use in the performance of some embodiments of the invention</i>		
<i>Gene Source</i>	<i>Expression Pattern</i>	<i>Reference</i>
Seed specific genes	seed	Simon, et al., Plant Mol. Biol. 5. 191, 1985; Scofield, et al., J. Biol. Chem. 262: 12202, 1987.; Baszczynski, et al., Plant Mol. Biol. 14: 633, 1990.
Brazil Nut albumin	seed	Pearson' et al., Plant Mol. Biol. 18: 235-

<i>Exemplary seed-preferred promoters for use in the performance of some embodiments of the invention</i>		
<i>Gene Source</i>	<i>Expression Pattern</i>	<i>Reference</i>
		245, 1992.
legumin	seed	Ellis, et al. Plant Mol. Biol. 10: 203-214, 1988
Glutelin (rice)	seed	Takaiwa, et al., Mol. Gen. Genet. 208: 15-22, 1986; Takaiwa, et al., FEBS Letts. 221: 43-47, 1987
Zein	seed	Matzke et al Plant Mol Biol, 143.323-32 1990
napA	seed	Stalberg, et al, Planta 199: 515-519, 1996
wheat LMW and HMW, glutenin-1	endosperm	Mol Gen Genet 216:81-90, 1989; NAR 17:461-2,
Wheat SPA	seed	Albanietal, Plant Cell, 9: 171- 184, 1997
wheat a, b and g gliadins	endosperm	EMBO3:1409-15, 1984
Barley ltrI promoter	endosperm	
barley B1, C, D hordein	endosperm	Theor Appl Gen 98:1253-62, 1999; Plant J 4:343-55, 1993; Mol Gen Genet 250:750-60, 1996
Barley DOF	endosperm	Mena et al, The Plant Journal, 116(1): 53-62, 1998
Biz2	endosperm	EP99106056.7
Synthetic promoter	endosperm	Vicente-Carabajosa et al., Plant J. 13: 629-640, 1998
rice prolamin NRP33	endosperm	Wu et al, Plant Cell Physiology 39(8) 885-889, 1998
rice -globulin Glb-1	endosperm	Wu et al, Plant Cell Physiology 39(8) 885-889, 1998
rice OSH1	embryo	Sato et al, Proc. Nati. Acad. Sci. USA, 93: 8117-8122
rice alpha-globulin REB/OHP-1	endosperm	Nakase et al. Plant Mol. Biol. 33: 513-S22, 1997
rice ADP-glucose PP	endosperm	Trans Res 6:157-68, 1997
maize ESR gene family	endosperm	Plant J 12:235-46, 1997
sorghum gammakafirin	endosperm	PMB 32:1029-35, 1996

<i>Exemplary seed-preferred promoters for use in the performance of some embodiments of the invention</i>		
Gene Source	Expression Pattern	Reference
KNOX	emryo	Postma-Haarsma et al, Plant Mol. Biol. 39:257-71, 1999
rice oleosin	Embryo and aleuton	Wu et al, J. Biochem., 123:386, 1998
sunflower oleosin	Seed (embryo and dry seed)	Cummins, et al., Plant Mol. Biol. 19: 873-876, 1992

Table III

<i>Exemplary flower-specific promoters for use in the performance of the invention</i>		
Gene Source	Expression Pattern	Reference
AtPRP4	flowers	www.sals. medium.edu/mg/tierney/html
chalene synthase (chsA)	flowers	Van der Meer, et al., Plant Mol. Biol. 15, 95-109, 1990.
LAT52	anther	Twell et al Mol. Gen Genet. 217:240-245 (1989)
apetala- 3	flowers	

Table IV

<i>Alternative rice promoters for use in the performance of the invention</i>		
PRO #	gene	expression
PR00001	Metallothionein Mte	transfer layer of embryo + calli
PR00005	putative beta-amylase	transfer layer of embryo
PR00009	Putative cellulose synthase	Weak in roots
PR00012	lipase (putative)	
PR00014	Transferase (putative)	
PR00016	peptidyl prolyl cis-trans isomerase (putative)	
PR00019	unknown	
PR00020	prp protein (putative)	
PR00029	noduline (putative)	
PR00058	Proteinase inhibitor Rgpi9	seed
PR00061	beta expansine EXPB9	Weak in young flowers
PR00063	Structural protein	young tissues+calli+embryo

<i>Alternative rice promoters for use in the performance of the invention</i>		
<i>PRO #</i>	<i>gene</i>	<i>expression</i>
PR00069	xylosidase (putative)	
PR00075	Prolamine 10Kda	strong in endosperm
PR00076	allergen RA2	strong in endosperm
PR00077	prolamine RP7	strong in endosperm
PR00078	CBP80	
PR00079	starch branching enzyme I	
PR00080	Metallothioneine-like ML2	transfer layer of embryo + calli
PR00081	putative caffeoyl- CoA 3-O methyltransferase	shoot
PR00087	prolamine RM9	strong in endosperm
PR00090	prolamine RP6	strong in endosperm
PR00091	prolamine RP5	strong in endosperm
PR00092	allergen RA5	
PR00095	putative methionine aminopeptidase	embryo
PR00098	ras-related GTP binding protein	
PR00104	beta expansine EXPB1	
PR00105	Glycine rich protein	
PR00108	metallothionein like protein (putative)	
PR00110	RCc3 strong root	
PR00111	uclacyanin 3-like protein	weak discrimination center / shoot meristem
PR00116	26S proteasome regulatory particle non-ATPase subunit 11	very weak meristem specific
PR00117	putative 40S ribosomal protein	weak in endosperm
PR00122	chlorophyll a/lo-binding protein precursor (Cab27)	very weak in shoot
PR00123	putative protochlorophyllide reductase	Strong leaves
PR00126	metallothionein RiCMT	strong discrimination center shoot meristem
PR00129	GOS2	Strong constitutive
PR00131	GOS9	
PR00133	chitinase Cht-3	very weak meristem specific
PR00135	alpha-globulin	Strong in endosperm
PR00136	alanine aminotransferase	Weak in endosperm

<i>Alternative rice promoters for use in the performance of the invention</i>		
<i>PRO #</i>	<i>gene</i>	<i>expression</i>
PR00138	Cvcllin A2	
PR00139	Cyclin D2	
PR00140	Cyclin D3	
PR00141	Cyclophyllin 2	Shoot and seed
PR00146	sucrose synthase SS1 (barley)	medium constitutive
PR00147	trypsin inhibitor ITR1 (barley)	weak in endosperm
PR00149	ubiquitine 2 with intron	strong constitutive
PR00151	WSI18	Embryo and stress
PR00156	HVA22 homologue (putative)	
PR00157	EL2	
PR00169	aquaporine	medium constitutive in young plants
PR00170	High mobility group protein	Strong constitutive
PR00171	reversibly glycosylated protein RGP1	weak constitutive
PR00173	cvtosolic MDH	shoot
PR00175	RAB21	Embryo and stress
PR00176	CDPK7	
PR00177	Cdc2-1	very weak in meristem
PR00197	sucrose synthase 3	
PRO0198	OsVP1	
PRO0200	OSH1	very weak in young plant meristem
PRO0208	putative chlorophyllase	
PRO0210	OsNRT1	
PRO0211	EXP3	
PR00216	phosphate transporter OjPT1	
PRO0218	oleosin 18kd	aleurone + embryo
PRO0219	ubiquitine 2 without intron	
PRO0220	RFL	
PRO0221	maize UBI delta intron	not detected
PR00223	glutelin-1	
PRO0224	fragment of prolamin RP6 promoter	
PRO0225	4xABRE	
PRO0226	glutelin OSGLUA3	

<i>Alternative rice promoters for use in the performance of the invention</i>		
<i>PRO #</i>	<i>gene</i>	<i>expression</i>
PRO0227	BLZ-2_short (barley)	
PR00228	BLZ-2_long (barley)	

[0072] According to a specific embodiment, the promoter utilized by the present invention is a strong constitutive promoter such that over expression of the construct inserts is effected following transformation. In certain embodiments, the promoter is the *Arabidopsis Actin 2* (ACT2) promoter (Accession No: U41998, see Table I above).

[0073] The nucleic acid construct of some embodiments of the invention can further include an appropriate selectable marker and/or an origin of replication. According to some embodiments of the invention, the nucleic acid construct utilized is a shuttle vector, which can propagate both in *E. coli* (wherein the construct comprises an appropriate selectable marker and origin of replication) and be compatible with propagation in cells. The construct according to the present invention can be, for example, a plasmid, a bacmid, a phagemid, a cosmid, a phage, a virus or an artificial chromosome.

[0074] The nucleic acid construct of some embodiments of the invention can be utilized to stably or transiently transform plant cells. In stable transformation, the nucleic acid is integrated into the plant genome and as such it represents a stable and inherited trait. In transient transformation, the exogenous polynucleotide is expressed by the cell transformed but it is not integrated into the genome and as such it represents a transient trait.

[0075] Thus, according to some aspects of the present invention, there is provided an isolated cell comprising the nucleic acid construct of the invention.

[0076] As used herein, the term "isolated cell" refers to a cell at least partially separated from a plant. In some embodiments, the isolated cell is a plant cell of a whole plant. The isolated cell is a plant cell, for example, a plant cell in culture.

[0077] The term "plant" as used herein encompasses whole plants, ancestors and progeny of the plants and plant parts, including seeds, shoots, stems, roots (including tubers), and plant cells, tissues and organs. The plant may be in any form including suspension cultures, embryos, meristematic regions, callus tissue, leaves, gametophytes, sporophytes, pollen, and microspores. Plants that are particularly useful in the methods of the invention include all plants which belong to the superfamily Viridiplantae, in particular monocotyledonous and dicotyledonous plants including a fodder or forage legume, ornamental plant, food crop, tree, or shrub selected from the list comprising *Acacia* spp., *Acer* spp., *Actinidia* spp., *Aesculus* spp., *Agathis australis*, *Albizia amara*, *Alsophila tricolor*, *Andropogon* spp., *Arachis* spp., *Areca catechu*, *Astelia fragrans*, *Astragalus cicer*, *Baikiaea plurijuga*, *Betula* spp., *Brassica* spp., *Bruguiera gymnorhiza*, *Burkea africana*, *Butea frondosa*, *Cadaba farinosa*, *Calliandra* spp.,

Camellia sinensis, Canna indica, Capsicum spp., Cassia spp., Centroema pubescens, Chacoomeles spp., Cinnamomum cassia, Coffea arabica, Colophospermum mopane, Coronilla varia, Cotoneaster serotina, Crataegus spp., Cucumis spp., Cupressus spp., Cyathea dealbata, Cydonia oblonga, Cryptomeria japonica, Cymbopogon spp., Cynthea dealbata, Cydonia oblonga, Dalbergia monetaria, Davallia divaricata, Desmodium spp., Dicksonia squarosa, Dibeteropogon amplectens, Dioclea spp., Dolichos spp., Dorycnium rectum, Echinochloa pyramidalis, Ehraffia spp., Eleusine coracana, Eragrestis spp., Erythrina spp., Eucalypfus spp., Euclea schimperi, Eulalia villosa, Pagopyrum spp., Feijoa sellowiana, Fragaria spp., Flemingia spp., Freycinetia banksli, Geranium thunbergii, GinAgo biloba, Glycine javanica, Gliricidia spp., Gossypium hirsutum, Grevillea spp., Guibourtia coleosperma, Hedsarum spp., Hemaffhia altissima, Heteropogon contortus, Hordeum vulgare, Hyparrhenia rufa, Hypericum erectum, Hypeffhelia dissolute, Indigo incamata, Iris spp., Leptarrhena pyrolifolia, Lespediza spp., Lettuce spp., Leucaena leucocephala, Loudetia simplex, Lotononis bainesli, Lotus spp., Macrotyloma axillare, Malus spp., Manihot esculenta, Medicago saliva, Metasequoia glyptostroboides, Musa sapientum, Nicotianum spp., Onobrychis spp., Ornithopus spp., Oryza spp., Peltophorum africanum, Pennisetum spp., Persea gratissima, Petunia spp., Phaseolus spp., Phoenix canariensis, Phormium cookianum, Photinia spp., Picea glauca, Pinus spp., Pisum sativum, Podocarpus totara, Pogonarthria fleckii, Pogonaffhria squarrosa, Populus spp., Prosopis cineraria, Pseudotsuga menziesii, Pterolobium stellatum, Pyrus communis, Quercus spp., Rhaphiolepis umbellata, Rhopalostylis sapida, Rhus natalensis, Ribes grossularia, Ribes spp., Robinia pseudoacacia, Rosa spp., Rubus spp., Salix spp., Schyzachyrium sanguineum, Sciadopitys vefficillata, Sequoia sempervirens, Sequoiadendron giganteum, Sorghum bicolor, Spinacia spp., Sporobolus fimbriatus, Stiburus alopecuroides, Stylosanthos humilis, Tadehagi spp., Taxodium distichum, Themeda triandra, Trifolium spp., Triticum spp., Tsuga heterophylla, Vaccinium spp., Vicia spp., Vitis vinifera, Watsonia pyramidata, Zantedeschia aethiopica, Zea mays, amaranth, artichoke, asparagus, broccoli, Brussels sprouts, cabbage, canola, carrot, cauliflower, celery, collard greens, flax, kale, lentil, oilseed rape, okra, onion, potato, rice, soybean, straw, sugar beet, sugar cane, sunflower, tomato, squash tea, maize, wheat, barely, rye, oat, peanut, pea, lentil and alfalfa, cotton, rapeseed, canola, pepper, sunflower, tobacco, eggplant, eucalyptus, a tree, an ornamental plant, a perennial grass and a forage crop. Alternatively algae and other non-Viridiplantae can be used for the methods of the present invention.

[0078] According to some embodiments of the invention, the plant or plant cell used by the method of the invention is a crop plant or cell of a crop plant such as rice, maize, wheat, barley, peanut, potato, sesame, olive tree, palm oil, banana, soybean, sunflower, canola, sugarcane, alfalfa, millet, leguminosae (bean, pea), flax, lupinus, rapeseed, tobacco, poplar and cotton.

[0079] According to further embodiments the plant cells include tobacco cells, *Agrobacterium rhizogenes* transformed root cell, celery cell, ginger cell, horseradish cell and carrot cells. In one embodiment the tobacco cells are from a tobacco cell line, such as, but not limited to *Nicotiana tabacum* L. cv Bright Yellow (BY-2) cells (Nagata et al, Int Rev Cytol 1992;132:1-30). The plant cells may be grown according to any type of suitable culturing method, including but not limited to, culture on a solid surface (such as a plastic culturing vessel or plate for example)

or in suspension. It will be noted that some cells, such as the BY-2 and carrot cells can be cultured and grown in suspension. Suitable devices and methods for culturing plant cells in suspension are known in the art, for example, as described in International Patent Applications PCT WO98/13469, WO2005/080544 and WO2007/010533. In yet another embodiment the cells are cells of whole tobacco plants or plant tissues, including, but not limited to *Nicotiana benthamiana*.

[0080] There are various methods of introducing foreign genes into both monocotyledonous and dicotyledonous plants (Potrykus, I., Annu. Rev. Plant. Physiol., Plant. Mol. Biol. (1991) 42:205-225; Shimamoto et al., Nature (1989) 338:274-276).

[0081] The principle methods of causing stable integration of exogenous DNA into plant genomic DNA include two main approaches:

1. (i) Agrobacterium-mediated gene transfer: Klee et al. (1987) Annu. Rev. Plant Physiol. 38:467-486; Klee and Rogers in Cell Culture and Somatic Cell Genetics of Plants, Vol. 6, Molecular Biology of Plant Nuclear Genes, eds. Schell, J., and Vasil, L. K., Academic Publishers, San Diego, Calif. (1989) p. 2-25; Gatenby, in Plant Biotechnology, eds. Kung, S. and Arntzen, C. J., Butterworth Publishers, Boston, Mass. (1989) p. 93-112.
2. (ii) Direct DNA uptake: Paszkowski et al., in Cell Culture and Somatic Cell Genetics of Plants, Vol. 6, Molecular Biology of Plant Nuclear Genes eds. Schell, J., and Vasil, L. K., Academic Publishers, San Diego, Calif. (1989) p. 52-68; including methods for direct uptake of DNA into protoplasts, Toriyama, K. et al. (1988) Bio/Technology 6:1072-1074. DNA uptake induced by brief electric shock of plant cells: Zhang et al. Plant Cell Rep. (1988) 7:379-384. Fromm et al. Nature (1986) 319:791-793. DNA injection into plant cells or tissues by particle bombardment, Klein et al. Bio/Technology (1988) 6:559-563; McCabe et al. Bio/Technology (1988) 6:923-926; Sanford, Physiol. Plant. (1990) 79:206-209; by the use of micropipette systems: Neuhaus et al., Theor. Appl. Genet. (1987) 75:30-36; Neuhaus and Spangenberg, Physiol. Plant. (1990) 79:213-217; glass fibers or silicon carbide whisker transformation of cell cultures, embryos or callus tissue, U.S. Pat. No. 5,464,765 or by the direct incubation of DNA with germinating pollen, DeWet et al. in Experimental Manipulation of Ovule Tissue, eds. Chapman, G. P. and Mantell, S. H. and Daniels, W. Longman, London, (1985) p. 197-209; and Ohta, Proc. Natl. Acad. Sci. USA (1986) 83:715-719.

[0082] The Agrobacterium system includes the use of plasmid vectors that contain defined DNA segments that integrate into the plant genomic DNA. Methods of inoculation of the plant tissue vary depending upon the plant species and the Agrobacterium delivery system. A widely used approach is the leaf disc procedure which can be performed with any tissue explant that provides a good source for initiation of whole plant differentiation. See, e.g., Horsch et al. in Plant Molecular Biology Manual A5, Kluwer Academic Publishers, Dordrecht (1988) p. 1-9. Another approach employs the Agrobacterium delivery system in combination with vacuum infiltration. The Agrobacterium system is especially viable in the creation of transgenic

dicotyledonous plants.

[0083] There are various methods of direct DNA transfer into plant cells. In electroporation, the protoplasts are briefly exposed to a strong electric field. In microinjection, the DNA is mechanically injected directly into the cells using very small micropipettes. In microparticle bombardment, the DNA is adsorbed on microprojectiles such as magnesium sulfate crystals or tungsten particles, and the microprojectiles are physically accelerated into cells or plant tissues.

[0084] Following stable transformation plant propagation is exercised. The most common method of plant propagation is by seed. Regeneration by seed propagation, however, has the deficiency that due to heterozygosity there is a lack of uniformity in the crop, since seeds are produced by plants according to the genetic variances governed by Mendelian rules. Basically, each seed is genetically different and each will grow with its own specific traits. Therefore, it is preferred that the transformed plant be produced such that the regenerated plant has the identical traits and characteristics of the parent transgenic plant. Therefore, it is preferred that the transformed plant be regenerated by micropropagation which provides a rapid, consistent reproduction of the transformed plants.

[0085] Micropropagation is a process of growing new generation plants from a single piece of tissue that has been excised from a selected parent plant or cultivar. This process permits the mass reproduction of plants having the preferred tissue expressing the fusion protein. The new generation plants which are produced are genetically identical to, and have all of the characteristics of, the original plant. Micropropagation allows mass production of quality plant material in a short period of time and offers a rapid multiplication of selected cultivars in the preservation of the characteristics of the original transgenic or transformed plant. The advantages of cloning plants are the speed of plant multiplication and the quality and uniformity of plants produced.

[0086] Micropropagation is a multi-stage procedure that requires alteration of culture medium or growth conditions between stages. Thus, the micropropagation process involves four basic stages: Stage one, initial tissue culturing; stage two, tissue culture multiplication; stage three, differentiation and plant formation; and stage four, greenhouse culturing and hardening. During stage one, initial tissue culturing, the tissue culture is established and certified contaminant-free. During stage two, the initial tissue culture is multiplied until a sufficient number of tissue samples are produced to meet production goals. During stage three, the tissue samples grown in stage two are divided and grown into individual plantlets. At stage four, the transformed plantlets are transferred to a greenhouse for hardening where the plants' tolerance to light is gradually increased so that it can be grown in the natural environment.

[0087] According to some embodiments of the invention, the transgenic plants are generated by transient transformation of leaf cells, meristematic cells or the whole plant.

[0088] Transient transformation can be effected by any of the direct DNA transfer methods

described above or by viral infection using modified plant viruses.

[0089] Viruses that have been shown to be useful for the transformation of plant hosts include CaMV, Tobacco mosaic virus (TMV), brome mosaic virus (BMV) and Bean Common Mosaic Virus (BV or BCMV). Transformation of plants using plant viruses is described in U.S. Pat. No. 4,855,237 (bean golden mosaic virus; BGV), EP-A 67,553 (TMV), Japanese Published Application No. 63-14693 (TMV), EPA 194,809 (BV), EPA 278,667 (BV); and Gluzman, Y. et al., Communications in Molecular Biology: Viral Vectors, Cold Spring Harbor Laboratory, New York, pp. 172-189 (1988). Pseudovirus particles for use in expressing foreign DNA in many hosts, including plants are described in WO 87/06261.

[0090] According to some embodiments of the invention, the virus used for transient transformations is avirulent and thus is incapable of causing severe symptoms such as reduced growth rate, mosaic, ring spots, leaf roll, yellowing, streaking, pox formation, tumor formation and pitting. A suitable avirulent virus may be a naturally occurring avirulent virus or an artificially attenuated virus. Virus attenuation may be effected by using methods well known in the art including, but not limited to, sub-lethal heating, chemical treatment or by directed mutagenesis techniques such as described, for example, by Kurihara and Watanabe (Molecular Plant Pathology 4:259-269, 2003), Gal-on et al. (1992), Atreya et al. (1992) and Huet et al. (1994).

[0091] Suitable virus strains can be obtained from available sources such as, for example, the American Type culture Collection (ATCC) or by isolation from infected plants. Isolation of viruses from infected plant tissues can be effected by techniques well known in the art such as described, for example by Foster and Tatlor, Eds. "Plant Virology Protocols: From Virus Isolation to Transgenic Resistance (Methods in Molecular Biology (Humana Pr), Vol 81)", Humana Press, 1998. Briefly, tissues of an infected plant believed to contain a high concentration of a suitable virus, preferably young leaves and flower petals, are ground in a buffer solution (e.g., phosphate buffer solution) to produce a virus infected sap which can be used in subsequent inoculations.

[0092] Construction of plant RNA viruses for the introduction and expression of non-viral nucleic acid sequences in plants is demonstrated by the above references as well as by Dawson, W. O. et al., Virology (1989) 172:285-292; Takamatsu et al. EMBO J. (1987) 6:307-311; French et al. Science (1986) 231:1294-1297; Takamatsu et al. FEBS Letters (1990) 269:73-76; and U.S. Pat. No. 5,316,931.

[0093] When the virus is a DNA virus, suitable modifications can be made to the virus itself. Alternatively, the virus can first be cloned into a bacterial plasmid for ease of constructing the desired viral vector with the foreign DNA. The virus can then be excised from the plasmid. If the virus is a DNA virus, a bacterial origin of replication can be attached to the viral DNA, which is then replicated by the bacteria. Transcription and translation of this DNA will produce the coat protein which will encapsidate the viral DNA. If the virus is an RNA virus, the virus is generally cloned as a cDNA and inserted into a plasmid. The plasmid is then used to make all

of the constructions. The RNA virus is then produced by transcribing the viral sequence of the plasmid and translation of the viral genes to produce the coat protein(s) which encapsidate the viral RNA.

[0094] In one embodiment, a plant viral polynucleotide is provided in which the native coat protein coding sequence has been deleted from a viral polynucleotide, a non-native plant viral coat protein coding sequence and a non-native promoter, preferably the subgenomic promoter of the non-native coat protein coding sequence, capable of expression in the plant host, packaging of the recombinant plant viral polynucleotide, and ensuring a systemic infection of the host by the recombinant plant viral polynucleotide, has been inserted. Alternatively, the coat protein gene may be inactivated by insertion of the non-native polynucleotide sequence within it, such that a protein is produced. The recombinant plant viral polynucleotide may contain one or more additional non-native subgenomic promoters. Each non-native subgenomic promoter is capable of transcribing or expressing adjacent genes or polynucleotide sequences in the plant host and incapable of recombination with each other and with native subgenomic promoters. Non-native (foreign) polynucleotide sequences may be inserted adjacent the native plant viral subgenomic promoter or the native and a non-native plant viral subgenomic promoters if more than one polynucleotide sequence is included. The non-native polynucleotide sequences are transcribed or expressed in the host plant under control of the subgenomic promoter to produce the desired products.

[0095] In a second embodiment, a recombinant plant viral polynucleotide is provided as in the first embodiment except that the native coat protein coding sequence is placed adjacent one of the non-native coat protein subgenomic promoters instead of a non-native coat protein coding sequence.

[0096] In a third embodiment, a recombinant plant viral polynucleotide is provided in which the native coat protein gene is adjacent its subgenomic promoter and one or more non-native subgenomic promoters have been inserted into the viral polynucleotide. The inserted non-native subgenomic promoters are capable of transcribing or expressing adjacent genes in a plant host and are incapable of recombination with each other and with native subgenomic promoters. Non-native polynucleotide sequences may be inserted adjacent the non-native subgenomic plant viral promoters such that the sequences are transcribed or expressed in the host plant under control of the subgenomic promoters to produce the desired product.

[0097] In a fourth embodiment, a recombinant plant viral polynucleotide is provided as in the third embodiment except that the native coat protein coding sequence is replaced by a non-native coat protein coding sequence.

[0098] The viral vectors are encapsidated by the coat proteins encoded by the recombinant plant viral polynucleotide to produce a recombinant plant virus. The recombinant plant viral polynucleotide or recombinant plant virus is used to infect appropriate host plants. The recombinant plant viral polynucleotide is capable of replication in the host, systemic spread in the host, and transcription or expression of foreign gene(s) (exogenous polynucleotide) in the

host to produce the desired protein.

[0099] Techniques for inoculation of viruses to plants may be found in Foster and Taylor, eds. "Plant Virology Protocols: From Virus Isolation to Transgenic Resistance (Methods in Molecular Biology (Humana Pr), Vol 81)", Humana Press, 1998; Maramorosh and Koprowski, eds. "Methods in Virology" 7 vols, Academic Press, New York 1967-1984; Hill, S.A. "Methods in Plant Virology", Blackwell, Oxford, 1984; Walkey, D.G.A. "Applied Plant Virology", Wiley, New York, 1985; and Kado and Agrawa, eds. "Principles and Techniques in Plant Virology", Van Nostrand-Reinhold, New York.

[0100] In addition to the above, the polynucleotide of the present invention can also be introduced into a chloroplast genome thereby enabling chloroplast expression.

[0101] A technique for introducing exogenous nucleic acid sequences to the genome of the chloroplasts is known. This technique involves the following procedures. First, plant cells are chemically treated so as to reduce the number of chloroplasts per cell to about one. Then, the exogenous polynucleotide is introduced via particle bombardment into the cells with the aim of introducing at least one exogenous polynucleotide molecule into the chloroplasts. The exogenous polynucleotides selected such that it is integratable into the chloroplast's genome via homologous recombination which is readily effected by enzymes inherent to the chloroplast. To this end, the nucleic acid sequence includes, in addition to a gene of interest, at least one polynucleotide stretch which is derived from the chloroplast's genome. In addition, the exogenous polynucleotide includes a selectable marker, which serves by sequential selection procedures to ascertain that all or substantially all of the copies of the chloroplast genomes following such selection will include the exogenous polynucleotide. Further details relating to this technique are found in U.S. Pat. Nos. 4,945,050; and 5,693,507 which are referred to herein. A polypeptide can thus be produced by the protein expression system of the chloroplast and become integrated into the chloroplast's inner membrane.

[0102] According to some embodiments of the invention, the method further comprises growing the plant cell expressing the nucleic acid. The plant cell can be grown as part of a whole plant, or, alternatively, in plant cell culture. In one embodiment, the plant cell is grown in axenic suspension culture, in a bioreactor. Bioreactors suitable for culturing the plant cells suspensions of the invention, and methods for growing the plant cells in suspension are described in detail in, *inter alia*, US Patent Nos. 6,391,638 and 7,951,557 and PCTs WO98/13469, WO2005/080544, WO2007/010533, WO2008/135991 and WO2008/132743, all of which are referred to herein.

[0103] Thus, the invention encompasses plants or plant cultures expressing the nucleic acid sequences, so as to produce the recombinant human α -galactosidase protein of the invention. Once expressed within the plant cell or the entire plant, the level of the human α -galactosidase protein encoded by the nucleic acid sequence can be determined by methods well known in the art such as, activity assays, Western blots using antibodies capable of specifically binding the human α -galactosidase protein, Enzyme-Linked Immuno Sorbent Assay (ELISA), radio-

immuno-assays (RIA), immunohistochemistry, immunocytochemistry, immunofluorescence and the like.

[0104] Methods of determining the level in the plant of the RNA transcribed from the nucleic acid sequence are well known in the art and include, for example, Northern blot analysis, reverse transcription polymerase chain reaction (RT-PCR) analysis (including quantitative, semi-quantitative or real-time RT-PCR) and RNA-*in situ* hybridization.

[0105] Glycan analysis of the expressed recombinant human α -galactosidase protein of the present invention indicates that the polypeptide is glycosylated in the plant cell, resulting in a recombinant human α -galactosidase protein having exposed mannose and plant specific glycan residues (see Example IV below). Thus, according to some embodiments of the invention, cells expressing the expression vector of the invention produce a human α -galactosidase protein having at least one, optionally at least two, optionally at least three or optionally at least four or more core β -(1,2) xylose residues. In yet other embodiments the cells expressing the expression vector of the invention produce a human α -galactosidase protein having at least one, optionally at least two, optionally at least three or optionally at least four or more core α -(1,3) fucose residues. In other embodiments the cells expressing the expression vector of the invention produce a human α -galactosidase protein having at least one, optionally at least two, optionally at least three or optionally at least four or more terminal β (1-2)N acetylglucosamine. In other embodiments the cells expressing the expression vector of the invention produce a human α -galactosidase protein having at least one, optionally at least two, optionally at least three or optionally at least four or more exposed mannose residues. In one particular embodiment the cells expressing the expression vector of the invention produce a human α -galactosidase protein having nine mannose residues (M9), at least one, optionally at least two, optionally at least three of them being exposed mannose residues. In one embodiment the cells expressing the expression vector of the invention produces a human α -galactosidase protein having at least one exposed mannose residue, at least one core β -(1,2) xylose residue and at least one α -(1,3) fucose residue.

[0106] Yet further, according to some embodiments of the invention, there is provided a composition comprising a plurality of human α -galactosidase protein molecules produced by cells expressing the expression vector of the invention, wherein the most predominant glycan structures of the plurality of human α -galactosidase protein molecules are mannose 3- β -(1,2) xylose- α -(1,3) fucose [Fc(3)M3X] and / or mannose 4- β -(1,2) xylose (M4X); and mannose 8 (M8). In some embodiments of the invention, the glycan structures mannose 3- β -(1,2) xylose- α -(1,3) fucose [Fc(3)M3X] and / or mannose 4- β -(1,2) xylose (M4X) comprise about 20%, about 25%, about 30%, about 35% or about 40%, about 45%, about 50%, about 55%, about 60%, about 70%, about 80% of the profile of human α -galactosidase protein molecules in the plurality, and the glycan structure mannose 8 (M8) comprises 20%, about 25%, about 30%, about 35% or about 40%, about 45%, about 50%, about 55%, about 60%, about 70%, about 80% of the profile of the human α -galactosidase protein molecules in the plurality.

[0107] In yet further embodiments of the present invention, the composition comprises a

plurality of human α -galactosidase protein molecules produced by cells expressing the expression vector of the invention, wherein said plurality of human α -galactosidase protein molecules comprise at least about 0.5%, at least about 0.6 %, about 0.7 %, about 0.8 %, about 0.9 %, about 1.0 %, about 1.1 %, about 1.2 %, about 1.3 %, about 1.4 %, about 1.5 %, about 1.75 %, about 2.0 %, about 2.5 %, about 3.0 %, about 3.5 %, about 4.0 %, about 4.5 %, about 5.0 % or more human α -galactosidase protein molecules having glycan structures of nine mannose residues, at least one, optionally at least two, optionally at least three of them being exposed mannose residues.

[0108] The human α -galactosidase protein produced by cells expressing the expression vector of the invention was shown to have α -galactosidase catalytic activity identical to that of native human α -galactosidase enzyme. Exposure of the recombinant α -galactosidase of the invention to conditions simulating an *in-vivo* environment of the enzyme when administered for enzyme replacement therapy further indicate that the human α -galactosidase protein produced by cells expressing the expression vector of the invention could possess pharmacokinetics similar to those of mammalian-cell expressed recombinant human α -galactosidase (Replagal®) (see Example 3 and Figs. 2 and 3 herein).

[0109] Thus, the human α -galactosidase protein produced by cells expressing the expression vector of the invention can be used to produce a pharmaceutical composition. The pharmaceutical composition can be used for treatment or prevention of Fabry disease in a subject in need thereof. In some embodiments, cells expressing the human α -galactosidase protein can be used for treatment or prevention of Fabry disease in a subject in need thereof. Methods for preparation and administration of plant cells recombinantly expressing human proteins are known in the art, for example, PCT WO2007/010533 to Shaaltiel et al.

[0110] Thus, according to another aspect of the present invention there is provided a pharmaceutical composition which includes, as an active ingredient thereof, a human α -galactosidase protein translationally fused at the C-terminal to an endoplasmic reticulum retention signal peptide, and a pharmaceutical acceptable carrier. In some embodiments of the present invention, the human α -galactosidase protein has an N-terminal Glycine residue. In some embodiments of the present invention, the human α -galactosidase protein has an amino acid sequence as set forth in SEQ ID NO: 16. In some embodiments, the human α -galactosidase protein translationally fused at the C-terminal to an endoplasmic reticulum retention signal peptide has an amino acid as set forth in SEQ ID NO: 21.

[0111] As used herein a "pharmaceutical composition" refers to a preparation of one or more of the active ingredients described herein with other chemical components such as physiologically suitable carriers and excipients. The purpose of a pharmaceutical composition is to facilitate administration of a compound to an organism.

[0112] Herein the term "active ingredient" refers to the recombinant human α -galactosidase protein accountable for the biological effect.

[0113] Hereinafter, the phrases "physiologically acceptable carrier" and "pharmaceutically acceptable carrier" which may be interchangeably used refer to a carrier or a diluent that does not cause significant irritation to an organism and does not abrogate the biological activity and properties of the administered compound. An adjuvant is included under these phrases.

[0114] Herein the term "excipient" refers to an inert substance added to a pharmaceutical composition to further facilitate administration of an active ingredient. Examples, without limitation, of excipients include calcium carbonate, calcium phosphate, various sugars and types of starch, cellulose derivatives, gelatin, vegetable oils and polyethylene glycols.

[0115] Techniques for formulation and administration of drugs may be found in "Remington's Pharmaceutical Sciences," Mack Publishing Co., Easton, PA, latest edition, which is incorporated herein by reference.

[0116] Suitable routes of administration may, for example, include oral, rectal, transmucosal, especially transnasal, intestinal or parenteral delivery, including intramuscular, subcutaneous and intramedullary injections as well as intrathecal, direct intraventricular, intracardiac, e.g., into the right or left ventricular cavity, into the common coronary artery, intravenous, intraperitoneal, intranasal, or intraocular injections.

[0117] Conventional approaches for drug delivery to the central nervous system (CNS) include: neurosurgical strategies (e.g., intracerebral injection or intracerebroventricular infusion); molecular manipulation of the agent (e.g., production of a chimeric fusion protein that comprises a transport peptide that has an affinity for an endothelial cell surface molecule in combination with an agent that is itself incapable of crossing the BBB) in an attempt to exploit one of the endogenous transport pathways of the BBB; pharmacological strategies designed to increase the lipid solubility of an agent (e.g., conjugation of water-soluble agents to lipid or cholesterol carriers); and the transitory disruption of the integrity of the BBB by hyperosmotic disruption (resulting from the infusion of a mannitol solution into the carotid artery or the use of a biologically active agent such as an angiotensin peptide). However, each of these strategies has limitations, such as the inherent risks associated with an invasive surgical procedure, a size limitation imposed by a limitation inherent in the endogenous transport systems, potentially undesirable biological side effects associated with the systemic administration of a chimeric molecule comprised of a carrier motif that could be active outside of the CNS, and the possible risk of brain damage within regions of the brain where the BBB is disrupted, which renders it a suboptimal delivery method.

[0118] Alternately, one may administer the pharmaceutical composition in a local rather than systemic manner, for example, via injection of the pharmaceutical composition directly into a tissue region of a patient.

[0119] The term "tissue" refers to part of an organism consisting of cells designed to perform a function or functions. Examples include, but are not limited to, brain tissue, retina, skin tissue, hepatic tissue, pancreatic tissue, bone, cartilage, connective tissue, blood tissue, muscle

tissue, cardiac tissue brain tissue, vascular tissue, renal tissue, pulmonary tissue, gonadal tissue, hematopoietic tissue.

[0120] Pharmaceutical compositions of some embodiments of the invention may be manufactured by processes well known in the art, e.g., by means of conventional mixing, dissolving, granulating, dragee-making, levigating, emulsifying, encapsulating, entrapping or lyophilizing processes.

[0121] Pharmaceutical compositions for use in accordance with some embodiments of the invention thus may be formulated in conventional manner using one or more physiologically acceptable carriers comprising excipients and auxiliaries, which facilitate processing of the active ingredients into preparations which, can be used pharmaceutically. Proper formulation is dependent upon the route of administration chosen.

[0122] For injection, the active ingredients of the pharmaceutical composition may be formulated in aqueous solutions, preferably in physiologically compatible buffers such as Hank's solution, Ringer's solution, or physiological salt buffer. For transmucosal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art.

[0123] For oral administration, the active ingredients can be optionally formulated through administration of the whole cells producing a human α -galactosidase protein according to the present invention. The active ingredients can also optionally be formulated by combining the active ingredients and/or the cells with pharmaceutically acceptable carriers well known in the art, producing pharmaceutical compositions. Such carriers enable the cells and/or pharmaceutical composition to be formulated as tablets, pills, dragees, capsules, liquids, gels, syrups, slurries, suspensions, and the like, for oral ingestion by a patient. When used for oral administration, whole cells, or fractions of cells can be use fresh or processed (e.g. lyophilized, frozen, dried, etc).

[0124] Pharmacological preparations for oral use can be made using a solid excipient, optionally grinding the resulting mixture, and processing the mixture of granules, after adding suitable auxiliaries if desired, to obtain tablets or dragee cores. Suitable excipients are, in particular, fillers such as sugars, including lactose, sucrose, mannitol, or sorbitol; cellulose preparations such as, for example, maize starch, wheat starch, rice starch, potato starch, gelatin, gum tragacanth, methyl cellulose, hydroxypropylmethylcellulose, sodium carbomethylcellulose; and/or physiologically acceptable polymers such as polyvinylpyrrolidone (PVP). If desired, disintegrating agents may be added, such as cross-linked polyvinyl pyrrolidone, agar, or alginic acid or a salt thereof such as sodium alginate.

[0125] Dragee cores are provided with suitable coatings. For this purpose, concentrated sugar solutions may be used which may optionally contain gum arabic, talc, polyvinyl pyrrolidone, carbopol gel, polyethylene glycol, titanium dioxide, lacquer solutions and suitable organic solvents or solvent mixtures. Dyestuffs or pigments may be added to the tablets or dragee

coatings for identification or to characterize different combinations of active compound doses.

[0126] Pharmaceutical compositions which can be used orally, include push-fit capsules made of gelatin as well as soft, sealed capsules made of gelatin and a plasticizer, such as glycerol or sorbitol. The push-fit capsules may contain the active ingredients in admixture with filler such as lactose, binders such as starches, lubricants such as talc or magnesium stearate and, optionally, stabilizers. In soft capsules, the active ingredients may be dissolved or suspended in suitable liquids, such as fatty oils, liquid paraffin, or liquid polyethylene glycols. In addition, stabilizers may be added. All formulations for oral administration should be in dosages suitable for the chosen route of administration.

[0127] For buccal administration, the compositions may take the form of tablets or lozenges formulated in conventional manner.

[0128] For administration by nasal inhalation, the active ingredients for use according to some embodiments of the invention are conveniently delivered in the form of an aerosol spray presentation from a pressurized pack or a nebulizer with the use of a suitable propellant, e.g., dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane or carbon dioxide. In the case of a pressurized aerosol, the dosage unit may be determined by providing a valve to deliver a metered amount. Capsules and cartridges of, e.g., gelatin for use in a dispenser may be formulated containing a powder mix of the compound and a suitable powder base such as lactose or starch.

[0129] The pharmaceutical composition described herein may be formulated for parenteral administration, e.g., by bolus injection or continuous infusion. Formulations for injection may be presented in unit dosage form, e.g., in ampoules or in multidose containers with optionally, an added preservative. The compositions may be suspensions, solutions or emulsions in oily or aqueous vehicles, and may contain formulatory agents such as suspending, stabilizing and/or dispersing agents.

[0130] Pharmaceutical compositions for parenteral administration include aqueous solutions of the active preparation in water-soluble form. Additionally, suspensions of the active ingredients may be prepared as appropriate oily or water based injection suspensions. Suitable lipophilic solvents or vehicles include fatty oils such as sesame oil, or synthetic fatty acids esters such as ethyl oleate, triglycerides or liposomes. Aqueous injection suspensions may contain substances, which increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol or dextran. Optionally, the suspension may also contain suitable stabilizers or agents which increase the solubility of the active ingredients to allow for the preparation of highly concentrated solutions.

[0131] Alternatively, the active ingredient may be in powder form for constitution with a suitable vehicle, e.g., sterile, pyrogen-free water based solution, before use.

[0132] The pharmaceutical composition of some embodiments of the invention may also be

formulated in rectal compositions such as suppositories or retention enemas, using, e.g., conventional suppository bases such as cocoa butter or other glycerides.

[0133] Pharmaceutical compositions suitable for use in context of some embodiments of the invention include compositions wherein the active ingredients are contained in an amount effective to achieve the intended purpose. More specifically, a therapeutically effective amount means an amount of active ingredients (a-galactosidase) effective to prevent, alleviate or ameliorate symptoms of a disorder (e.g., Fabry's disease) or prolong the survival of the subject being treated.

[0134] Determination of a therapeutically effective amount is well within the capability of those skilled in the art, especially in light of the detailed disclosure provided herein.

[0135] For any preparation used in the methods of the invention, the therapeutically effective amount or dose can be estimated initially from in vitro and cell culture assays. For example, a dose can be formulated in animal models to achieve a desired concentration or titer. Such information can be used to more accurately determine useful doses in humans.

[0136] Toxicity and therapeutic efficacy of the active ingredients described herein can be determined by standard pharmaceutical procedures in vitro, in cell cultures or experimental animals. The data obtained from these in vitro and cell culture assays and animal studies can be used in formulating a range of dosage for use in human. The dosage may vary depending upon the dosage form employed and the route of administration utilized. The exact formulation, route of administration and dosage can be chosen by the individual physician in view of the patient's condition. (See e.g., Fingl, et al., 1975, in "The Pharmacological Basis of Therapeutics", Ch. 1 p.1).

[0137] Dosage amount and interval may be adjusted individually to provide serum and cell levels of the active ingredient which are sufficient to induce or suppress the biological effect (minimal effective concentration, MEC). The MEC will vary for each preparation, but can be estimated from in vitro data. Dosages necessary to achieve the MEC will depend on individual characteristics and route of administration. Detection assays can be used to determine plasma concentrations.

[0138] Depending on the severity and responsiveness of the condition to be treated, dosing can be of a single or a plurality of administrations, with course of treatment lasting from several days to several weeks or until cure is effected or diminution of the disease state is achieved.

[0139] The amount of a composition to be administered will, of course, be dependent on the subject being treated, the severity of the affliction, the manner of administration, the judgment of the prescribing physician, etc.

[0140] Compositions of some embodiments of the invention may, if desired, be presented in a pack or dispenser device, such as an FDA approved kit, which may contain one or more unit

dosage forms containing the active ingredient. The pack may, for example, comprise metal or plastic foil, such as a blister pack. The pack or dispenser device may be accompanied by instructions for administration. The pack or dispenser may also be accommodated by a notice associated with the container in a form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals, which notice is reflective of approval by the agency of the form of the compositions or human or veterinary administration. Such notice, for example, may be of labeling approved by the U.S. Food and Drug Administration for prescription drugs or of an approved product insert. Compositions comprising a preparation of the invention formulated in a compatible pharmaceutical carrier may also be prepared, placed in an appropriate container, and labeled for treatment of an indicated condition, as is further detailed above.

[0141] The pharmaceutical composition can be used for treatment or prevention of Fabry disease in a subject in need thereof. Thus, according to another aspect of the present invention there is provided a method for treating or preventing Fabry disease in a subject in need thereof, the method comprising administering to the subject an effective amount of a pharmaceutical composition which includes, as an active ingredient thereof, a human α -galactosidase protein translationally fused at the C-terminal to an endoplasmic reticulum retention signal peptide, and a pharmaceutical acceptable carrier. In some embodiments of the present invention, the human α -galactosidase protein has an N-terminal Glycine residue. In other embodiments, the human α -galactosidase protein has an amino acid sequence as set forth in SEQ ID NO: 16.

[0142] The term "treating" refers to inhibiting, preventing or arresting the development of a pathology (disease, disorder or condition) and/or causing the reduction, remission, or regression of a pathology. Those of skill in the art will understand that various methodologies and assays can be used to assess the development of a pathology, and similarly, various methodologies and assays may be used to assess the reduction, remission or regression of a pathology.

[0143] As used herein, the term "preventing" refers to keeping a disease, disorder or condition from occurring in a subject who may be at risk for the disease, but has not yet been diagnosed as having the disease.

[0144] As used herein, the term "subject" includes mammals, preferably human beings at any age which suffer from the pathology. Preferably, this term encompasses individuals who are at risk to develop the pathology.

[0145] As used herein the phrase "treatment regimen" refers to a treatment plan that specifies the type of treatment, dosage, schedule and/or duration of a treatment provided to a subject in need thereof (e.g., a subject diagnosed with a pathology). The selected treatment regimen can be an aggressive one which is expected to result in the best clinical outcome (e.g., complete cure of the pathology) or a more moderate one which may relieve symptoms of the pathology yet results in incomplete cure of the pathology. It will be appreciated that in certain cases the

more aggressive treatment regimen may be associated with some discomfort to the subject or adverse side effects (e.g., a damage to healthy cells or tissue). The type of treatment can include a surgical intervention (e.g., removal of lesion, diseased cells, tissue, or organ), a cell replacement therapy, an administration of a therapeutic drug (e.g., receptor agonists, antagonists, hormones, chemotherapy agents) in a local or a systemic mode, an exposure to radiation therapy using an external source (e.g., external beam) and/or an internal source (e.g., brachytherapy) and/or any combination thereof. The dosage, schedule and duration of treatment can vary, depending on the severity of pathology and the selected type of treatment, and those of skills in the art are capable of adjusting the type of treatment with the dosage, schedule and duration of treatment.

[0146] It is expected that during the life of a patent maturing from this application many relevant vectors, promoter elements, plant cells and carriers will be developed and the scope of the terms provided herein is intended to include all such new technologies *a priori*.

[0147] As used herein the term "about" refers to $\pm 10\%$.

[0148] The terms "comprises", "comprising", "includes", "including", "having" and their conjugates mean "including but not limited to".

[0149] The term "consisting of means "including and limited to".

[0150] The term "consisting essentially of" means that the composition, method or structure may include additional ingredients, steps and/or parts, but only if the additional ingredients, steps and/or parts do not materially alter the basic and novel characteristics of the claimed composition, method or structure.

[0151] As used herein, the singular form "a", "an" and "the" include plural references unless the context clearly dictates otherwise. For example, the term "a compound" or "at least one compound" may include a plurality of compounds, including mixtures thereof.

[0152] Throughout this application, various embodiments of this invention may be presented in a range format. It should be understood that the description in range format is merely for convenience and brevity and should not be construed as an inflexible limitation on the scope of the invention. Accordingly, the description of a range should be considered to have specifically disclosed all the possible subranges as well as individual numerical values within that range. For example, description of a range such as from 1 to 6 should be considered to have specifically disclosed subranges such as from 1 to 3, from 1 to 4, from 1 to 5, from 2 to 4, from 2 to 6, from 3 to 6 etc., as well as individual numbers within that range, for example, 1, 2, 3, 4, 5, and 6. This applies regardless of the breadth of the range.

[0153] Whenever a numerical range is indicated herein, it is meant to include any cited numeral (fractional or integral) within the indicated range. The phrases "ranging/ranges between" a first indicate number and a second indicate number and "ranging/ranges from" a

first indicate number "to" a second indicate number are used herein interchangeably and are meant to include the first and second indicated numbers and all the fractional and integral numerals therebetween.

[0154] As used herein the term "method" refers to manners, means, techniques and procedures for accomplishing a given task including, but not limited to, those manners, means, techniques and procedures either known to, or readily developed from known manners, means, techniques and procedures by practitioners of the chemical, pharmacological, biological, biochemical and medical arts.

[0155] As used herein, the term "treating" includes abrogating, substantially inhibiting, slowing or reversing the progression of a condition, substantially ameliorating clinical or aesthetical symptoms of a condition or substantially preventing the appearance of clinical or aesthetical symptoms of a condition.

[0156] It is appreciated that certain features of the invention, which are, for clarity, described in the context of separate embodiments, may also be provided in combination in a single embodiment. Conversely, various features of the invention, which are, for brevity, described in the context of a single embodiment, may also be provided separately or in any suitable subcombination or as suitable in any other described embodiment of the invention. Certain features described in the context of various embodiments are not to be considered essential features of those embodiments, unless the embodiment is inoperative without those elements.

[0157] Various embodiments and aspects of the present invention as delineated hereinabove and as claimed in the claims section below find experimental support in the following examples.

EXAMPLES

[0158] Reference is now made to the following examples, which together with the above descriptions illustrate some embodiments of the invention in a non limiting fashion.

[0159] Generally, the nomenclature used herein and the laboratory procedures utilized in the present invention include molecular, biochemical, microbiological and recombinant DNA techniques. Such techniques are thoroughly explained in the literature. See, for example, "Molecular Cloning: A laboratory Manual" Sambrook et al., (1989); "Current Protocols in Molecular Biology" Volumes I-III Ausubel, R. M., ed. (1994); Ausubel et al., "Current Protocols in Molecular Biology", John Wiley and Sons, Baltimore, Maryland (1989); Perbal, "A Practical Guide to Molecular Cloning", John Wiley & Sons, New York (1988); Watson et al., "Recombinant DNA", Scientific American Books, New York; Birren et al. (eds) "Genome Analysis: A Laboratory Manual Series", Vols. 1-4, Cold Spring Harbor Laboratory Press, New York (1998); methodologies as set forth in U.S. Pat. Nos. 4,666,828; 4,683,202; 4,801,531; 5,192,659 and 5,272,057; "Cell Biology: A Laboratory Handbook", Volumes I-III Cellis, J. E., ed. (1994); "Culture of Animal Cells - A Manual of Basic Technique" by Freshney, Wiley-Liss, N. Y.

(1994), Third Edition; "Current Protocols in Immunology" Volumes I-III Coligan J. E., ed. (1994); Stites et al. (eds), "Basic and Clinical Immunology" (8th Edition), Appleton & Lange, Norwalk, CT (1994); Mishell and Shiigi (eds), "Selected Methods in Cellular Immunology", W. H. Freeman and Co., New York (1980); available immunoassays are extensively described in the patent and scientific literature, see, for example, U.S. Pat. Nos. 3,791,932; 3,839,153; 3,850,752; 3,850,578; 3,853,987; 3,867,517; 3,879,262; 3,901,654; 3,935,074; 3,984,533; 3,996,345; 4,034,074; 4,098,876; 4,879,219; 5,011,771 and 5,281,521; "Oligonucleotide Synthesis" Gait, M. J., ed. (1984); "Nucleic Acid Hybridization" Hames, B. D., and Higgins S. J., eds. (1985); "Transcription and Translation" Hames, B. D., and Higgins S. J., eds. (1984); "Animal Cell Culture" Freshney, R. I., ed. (1986); "Immobilized Cells and Enzymes" IRL Press, (1986); "A Practical Guide to Molecular Cloning" Perbal, B., (1984) and "Methods in Enzymology" Vol. 1, 2, 317, Academic Press; "PCR Protocols: A Guide To Methods And Applications", Academic Press, San Diego, CA (1990); Marshak et al., "Strategies for Protein Purification and Characterization - A Laboratory Course Manual" CSHL Press (1996).

[0160] Other general references are provided throughout this document. The procedures therein are believed to be well known in the art and are provided for the convenience of the reader.

EXAMPLE I: CONSTRUCTION OF THE PLANT α -GALACTOSIDASE EXPRESSION CONSTRUCT

[0161] cDNA encoding the human α -galactosidase protein (EC 3.2.1-22 GenBank: X05790) was optimized and synthesized by GENEART AG (Regensburg, Germany). The codon usage without the leader peptide (endoplasmic reticulum target signal peptide) was adapted to the codon bias of *Nicotiana tabacum* genes. During the optimization process the following cis-acting sequence motifs were avoided: Internal TATA-boxes, chi-sites and ribosomal entry sites, AT-rich or GC-rich sequence stretches, RNA instability elements ("killer motifs"), Repeat sequences and RNA secondary structures, splice donor (cryptic) and acceptor sites, branch points. In addition, regions of very high (>80%) or very low (<30%) GC content has been avoided.

[0162] The nucleotide sequence of the native human α -galactosidase leader peptide (endoplasmic reticulum target signal peptide) (Figure 1, first 31 amino acids in red) of full length human α -galactosidase protein (GenBank: X05790) was replaced with a nucleotide sequence encoding the 33 amino acid endoplasmic reticulum targeting signal peptide (leader peptide) of the Arabidopsis ABPI protein (marked in green in Figure 1, SEQ ID NO: 4). This signal peptide provides efficient targeting of α -galactosidase to the secretory pathway and is cleaved from the polypeptide, by signal peptidase, once the protein has been translocated into the endoplasmic reticulum. A nucleotide sequence (SEQ ID NO: 19) encoding the endoplasmic reticulum retention signal SEKDEL (SEQ ID NO: 6) was added to the cDNA sequence at the 3' terminus, allowing retrieval of the expressed protein from the Golgi apparatus, effectively maintaining the protein in the endoplasmic reticulum. SEQ ID NO: 2 represents the complete

coding sequence of the expressed recombinant human α -galactosidase (SEQ ID NO: 1), including the N-terminal ABPI endoplasmic reticulum targeting signal peptide, human α -galactosidase protein and the SEKDEL endoplasmic reticulum retention signal.

EXAMPLE II: EXPRESSION OF RECOMBINANT HUMAN α -GALACTOSIDASE IN PLANTS

Transient expression system in *N.benthamiana*

[0163] The use of plant viral vectors was chosen in this case as an alternative to transgenic plants, allowing for the rapid, high level transient expression of proteins in mature whole plants.

[0164] The protein of interest was expressed from a strong subgenomic viral coat protein promoter. The system relies on transient amplification (by agroinfection) of viral vectors delivered to a plant by *Agrobacterium*, in which the plant functional promoter and the cDNA encoding a viral replicon are transferred as T-DNA from *agrobacterium* into plant cells. The T-DNA is transcribed *in-planta* by the plant promoter to generate biologically active viral RNA that initiates self replication.

[0165] For the transient expression a 3 vector recombination system based on the system previously developed as described (Gleba et al., Vaccine 23 2042-2048, 2005) was employed, α -galactosidase cDNA was inserted into one of the vectors, and the two other vectors containing genes for construction of the whole viral replicon (RdRp and Integrase), thus generating a biologically active viral RNA capable of initiating self replication.

Transfection of whole plants-

[0166] *N. Benthamiana* plants were germinated and grown in commercial mix soil (Givaat Ada, IL) supplemented with granular slow release fertilizer (Scott Marysville, OH) under a long day (16h light / 8h dark) light regime at 24°C-25°C.

[0167] Agrobacteria were transformed with the pICH20866-alpha-GAL based replicon vector system using electroporation (2500V, 5msec) [den Dulk-Ra, A. and Hooykaas, P.J. (1995) Methods Mol. Biol. 55:63-72]. Plants were infiltrated with *Agrobacteria* containing the 3 ICON plasmids by vacuum infiltration with standard methods known in the art. Briefly, *N. benthamiana* plants, 5-6 week old were infiltrated by immersing all aerial plant organs into a bacterial suspension and were placed in a vacuum chamber. A minus (-) 0.8 bar vacuum was applied for 1 minute, followed by a quick return to atmospheric pressure. Plants were returned to the greenhouse for additional 5-7 days under the same growth conditions.

Tobacco Plant Extract:

[0168] Samples of *Nicotiana benthamiana* leaves were harvested 5 days post infiltration and extracted in Laemmli buffer for SDS-PAGE, or in activity assay buffer (20mM citric acid, 30 mM sodium phosphate, 0.1%BSA and 0.67% ethanol, pH 4.6.) for assay of catalytic activity of the plant expressed protein.

Tobacco Plant Extract Purification:

[0169] Human α -galactosidase protein from plant extracts was purified by a two-step ammonium sulfate differential precipitation ("salting out": 1st step 0.57M; 2nd step 2.27M), followed by hydrophobic interaction chromatography (Phenyl 650 M resin) and cation exchange chromatography.

Stable expression in *N. tobaccum* BY2 cells

[0170] Agrobacterium mediated transformation is widely used to introduce foreign genes into a plant cell genome. This technique is based on the natural capability of the agrobacterium to transform plant cells by transferring a plasmid DNA segment, the transferred DNA (T-DNA), into the host cell genome. Using this approach, a T-DNA molecule, consisting of a foreign gene and its regulatory elements, is randomly introduced into the plant genome. The site of integration, as well as the copy number of the gene insertions is not controlled, thus the transformation process results in a 'pool' of transgenic cells composed of cells with various levels of expression of the transgene. The transgenic 'pool' is subsequently used for clone isolation. Clone isolation results in the establishment of many single cell lines, from which the clone with the highest expression level of the foreign gene is then selected.

[0171] BY2 suspension culture was co-cultivated, for 48 hours, with the Agrobacterium tumefaciens EHA105 strain carrying the vector harboring the α -GAL gene and the neomycin phosphotransferase (NPTII) selection gene.

[0172] Subsequently, the cells were kept in media supplemented with 50mg/L of Kanamycin and 250mg/L Cefotaxime. The NPTII gene confers resistance to Kanamycin, thus only NPTII positive BY2 cells survive in this selection media. The Cefotaxime was used to selectively kill the agrobacterium, the plant cells being resistant to this antibiotic. Once a nicely growing transgenic cell suspension was established, it was used for screening and isolating individual cell lines. To allow for the selection of individual cell lines, aliquots of highly diluted cell suspension were spread on solid BY-2 medium. The cells were then grown until small calli developed. Each callus was then re-suspended in liquid culture. The cells were then sampled and the samples were evaluated for α -GAL levels. The lines that expressed relatively high α -GAL activity levels were then further re-analyzed and compared for α -GAL levels ending with

the final selection of candidate α -GAL expressing lines.

Tobacco Cell Extract:

[0173] 100 mg of transformed BY2 cells expressing the human α -galactosidase protein were homogenized for 5min in 200ul 20mM phosphate buffer 0.5M containing 20mM EDTA 2mM DTT and 2mM PMSF, pH=7.2. The homogenate was centrifuged and the supernatant containing the recombinant human α -galactosidase protein was collected [crude extract]. α -galactosidase catalytic activity in crude was determined by PNP-G assay.

PNP-G Assay

[0174] α -galactosidase galactoside hydrolase activity was determined using p-nitrophenylalpha-D-galactopyranoside (pNP-alpha-D-Gal, GBB1290, IRIS Biotech, Germany) as a hydrolysis substrate. The assay buffer contained 20 mM citric acid, 30 mM sodium phosphate, 0.1 % BSA and 0.67 % ethanol at pH 4.6. Samples (50 μ l) were incubated with 150 μ l assay buffer. The substrate (pNP-alpha-D-Gal) was added (30 μ L) to a final concentration of 8 mM p-NP-alpha-D Gal. The reaction mixture was incubated at 37°C for 90 minutes. After 90 minutes, the reaction was quenched by the addition of 100 μ l sodium carbonate (2M) to each well, in order to terminate the reaction and enable the generation of the p-nitrophenolate chromophore. Results were calculated from Absorbance at 405 nm was measured.

[0175] **Modification of the plant-expressed human α -galactosidase:** Sequencing of the protein product from the transformed plants revealed that, in some instances, upon cleavage of the ABPI signal, the recombinant human α -galactosidase protein retains the last amino acid Glycine of the signal peptide at the N terminus of the mature α -galactosidase protein. Thus, the recombinant human α -galactosidase protein can be either without the Glycine of the signal peptide at the N-terminus (e.g. SEQ ID NO: 20, coding sequence as in SEQ ID NO: 22) or including a Glycine at the N- terminus (e.g. SEQ ID NO: 21, coding sequence as in SEQ ID NO: 23).

[0176] **Glycan analysis of the plant-expressed human α -galactosidase:** Samples of recombinant human α -galactosidase protein product from the transformed plants were reduced, alkylated with DTT and iodoacetamide, and separated on a 12.5% SDS-PAGE. Bands corresponding to the correct molecular weight were excised, protein extracted, and subjected to glycan analysis consisting of trypsin digestion followed by both PNGase A and PNGase F digestion. PNGase A digestion releases all the N-linked glycans and PNGase F release all glycans except those containing a 1-3 core fucose.

[0177] The free glycans were extracted, purified and then labeled with the fluorescent reagent anthranilamide (2AB) followed by removal of excess 2AB.

[0178] Glycans were separated using a TSK gel Amide 80 normal phase HPLC and detected using a fluorescence detector. A Dextran hydrolysate served as a ladder for calculation of glucose unit (GU) values. Glycan profile is constructed by calculating the relative peak areas from the chromatogram of the PNGase A digestion. Assignment of the glycans is established by calculation of the GU values of the peaks found in both endoglycoside digestions and based on additional various exoglycoside digestions.

Results:

[0179] Comparing between different α -galactosidase constructs, it was uncovered that the addition of an ABPI endoplasmic reticulum targeting signal peptide (SEQ ID NO: 4) to the human recombinant α -galactosidase polypeptide improved expression of the recombinant protein in transiently expressing *N. benthamiana* plants (Figure 2, lane 2), compared to α -galactosidase sequences expressed with an apple pectinase endoplasmic reticulum targeting signal peptide (apple pectinase ER targeting signal peptide as encoded by SEQ ID NO: 10) (α -galactosidase-apple pectinase ER signal peptide-protein as encoded by SEQ ID NO: 8 or SEQ ID NO: 11), or α -galactosidase protein expressed with an endochitinase B endoplasmic reticulum targeting signal peptide (endochitinase signal protein, SEQ ID NO: 14, as encoded by SEQ ID NO: 15). Assay of the catalytic activity (see above) confirmed that increased protein seen in Figure 2 is reflected in concomitant increase in enzymatic activity (Figure 3, column B) per kg leaves, compared with α -galactosidase sequences expressed with an apple pectinase endoplasmic reticulum targeting signal peptide or α -galactosidase protein expressed with an endochitinase B endoplasmic reticulum targeting signal peptide (endochitinase signal protein, SEQ ID NO: 14, as encoded by SEQ ID NO: 15).

[0180] Glycan analysis of the recombinant human α -galactosidase protein product from the transformed tobacco cells expressing a human α -galactosidase protein with the addition of an ABPI endoplasmic reticulum targeting signal peptide (e.g. SEQ ID NO: 1, encoded by SEQ ID NO: 2) revealed a glycan profile comprising main glycan structures of high mannose glycan structures (e.g. 3 to 6, 7, 8 or 9 mannose residues, with terminal, exposed mannose), and characteristic plant-specific glycans, such as β -(1,2) xylose and α -(1,3) fucose (Figure 7). Predominant glycan structures representing a high percentage of the glycan profile included about 30% having mannose 3- β -(1,2) xylose- α -(1,3) fucose [Fc(3)M3X] and/or mannose 4- α -(1,2) xylose (M4X); and another about 30% mannose 8 (M8) glycans (Figure 8). Figure 8 is an illustrated table showing the distribution of the glycan structures within the protein samples from the transformed cells. A-Gal A and A-Gal-B are two representative analyses of the recombinant human α -galactosidase protein product from the transformed plant cells.

[0181] Glycan structures can be further categorized according to the sugar residues present in the complex glycans. Table V below details the distribution of glycan structures in the human α -galactosidase protein product from transformed plants, according to specific sugars:

Table V-Saccharide content of glycans

GLYCAN TYPE	% OF TOTAL GLYCANS
Fucose containing glycans	~35
Xylose containing glycans	~50
High mannose	~60
N-acetylglucosamine containing glycans	~10

[0182] Thus, the human α -galactosidase protein product expressed in tobacco cell culture from the construct comprising SEQ ID NO: 2 was characterized by significant percentage of plant specific glycan structures (having fucose and xylose components), and high mannose glycosylation.

EXAMPLE III- STABILITY OF PLANT EXPRESSED HUMAN RECOMBINANT α -GALACTOSIDASE UNDER IN- VIVO CONDITIONS

[0183] In order to approximate pharmacokinetics of the plant expressed human recombinant α -galactosidase, recombinant enzyme preparations were exposed to simulated *in-vivo* conditions and catalytic activity monitored.

Stability in plasma:

[0184] Stability of enzymatic activity in plasma was assessed by adding recombinant plant extract or α -galactosidase purified from plant extract to human plasma to a final concentration of 1 μ g/ml (e.g. 10 μ l extract or purified α -galactosidase to 190 μ l human plasma), incubating at 37 °C, and following the α -galactosidase catalytic activity in samples at set intervals over an hour's time.

[0185] Comparison between α -galactosidase from *N. benthamiana* plants transformed with SEQ ID NO: 11, encoding an α -galactosidase with an apple pectinase endoplasmic reticulum targeting signal peptide (SEQ ID NO: 12) and α -galactosidase from *N. benthamiana* plants transformed with SEQ ID NO: 2, encoding α -galactosidase with an ABPI endoplasmic reticulum targeting signal peptide (SEQ ID NO: 1) (Figure 4) indicates a clear advantage of the *N. benthamiana*-expressed α -galactosidase from the construct having an ABPI endoplasmic reticulum targeting signal peptide (Figure 4, closed or solid squares), as compared to recombinant α -galactosidase expressed from the construct with an apple pectinase endoplasmic reticulum targeting signal leader (Figure 4, open squares).

[0186] Comparison between α -galactosidase expressed in *N. tabacum* plant cells (BY2) transformed with SEQ ID NO: 2, encoding an α -galactosidase protein with an ABPI endoplasmic reticulum targeting signal peptide (SEQ ID NO: 1), resulting in a mature

recombinant α -galactosidase protein as set forth in SEQ ID NO: 21 with α -galactosidase expressed in mammalian cells (REPLAGAL™) revealed similar stability of the enzymatic activity when incubated for one hour at 37 °C in plasma (Figure 5), and when incubated over 9 days under lysosome-like conditions (pH=4.6 and 37 °C, Figure 6). This similarity under simulated *in-vivo* conditions suggests that the plant cell expressed recombinant α -galactosidase would have pharmacokinetics comparable to those of the mammalian cell-expressed, clinically approved enzyme (REPLAGAL™).

[0187] In addition, citation or identification of any reference in this application shall not be construed as an admission that such reference is available as prior art to the present invention. To the extent that section headings are used, they should not be construed as necessarily limiting.

SEQUENCE LISTING

[0188]

<110> Protalix Ltd. Shulman, Avidor Hanania, Uri Kizhner, Tali Shaaltiel, Yosef

<120> NUCLEIC ACID CONSTRUCT FOR EXPRESSION OF ALPHA-GALACTOSIDASE IN PLANTS AND PLANT CELLS

<130> 51336

<150> PCT/IL2011/000209

<151> 2011-03-02

<150> US 61/434,499

<151> 2011-01-20

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<151> 2011-01-20

<160> 23

<170> PatentIn version 3.5

<210> 1

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Trp Glu Arg Phe Met Cys Asn Leu Asp Cys Gln Glu Glu Pro Asp Ser
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Cys Ile Ser Glu Lys Leu Phe Met Glu Met Ala Glu Leu Met Val Ser
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Glu Gly Trp Lys Asp Ala Gly Tyr Glu Tyr Leu Cys Ile Asp Asp Cys
85 90 95

Trp Met Ala Pro Gln Arg Asp Ser Glu Gly Arg Leu Gln Ala Asp Pro
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Gln Arg Phe Pro His Gly Ile Arg Gln Leu Ala Asn Tyr Val His Ser
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Lys Gly Leu Lys Leu Gly Ile Tyr Ala Asp Val Gly Asn Lys Thr Cys
 130 135 140

Ala Gly Phe Pro Gly Ser Phe Gly Tyr Tyr Asp Ile Asp Ala Gln Thr
 145 150 155 160

Phe Ala Asp Trp Gly Val Asp Leu Leu Lys Phe Asp Gly Cys Tyr Cys
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180 185 190

Leu Asn Arg Thr Gly Arg Ser Ile Val Tyr Ser Cys Glu Trp Pro Leu
195 200 205

Tyr Met Trp Pro Phe Gln Lys Pro Asn Tyr Thr Glu Ile Arg Gln Tyr
210 215 220

Cys Asn His Trp Arg Asn Phe Ala Asp Ile Asp Asp Ser Trp Lys Ser
225 230 235 240

Ile Lys Ser Ile Leu Asp Trp Thr Ser Phe Asn Gln Glu Arg Ile Val
245 250 255

Asp Val Ala Gly Pro Gly Gly Trp Asn Asp Pro Asp Met Leu Val Ile
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290

295

300

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Val Ala Met Ile Asn Arg Gln Glu Ile Gly Gly Pro Arg Ser Tyr Thr

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360

365

Ile Ala Val Ala Ser Leu Gly Lys Gly Val Ala Cys Asn Pro Ala Cys
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Phe Ile Thr Gln Leu Leu Pro Val Lys Arg Lys Leu Gly Phe Tyr Glu
 385 390 395 400

Trp Thr Ser Arg Leu Arg Ser His Ile Asn Pro Thr Gly Thr Val Leu
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 (ABPI+AGAL+SEKDEL)

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<213> Homo sapiens

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			20			25						30			

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Gly	Trp	Lys	Asp	Ala	Gly	Tyr	Glu	Tyr	Leu	Cys	Ile	Asp	Asp	Cys	Trp
	50				55				60						

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<212> DNA

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Cys Asn Leu Asp Cys Gln Glu Glu Pro Asp Ser Cys Ile Ser Glu Lys			
50	55	60	
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Leu Phe Met Glu Met Ala Glu Leu Met Val Ser Glu Gly Trp Lys Asp			
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75	80		

Ala Gly Tyr Glu Tyr Leu Cys Ile Asp Asp Cys Trp Met Ala Pro Gln			
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Arg Asp Ser Glu Gly Arg Leu Gln Ala Asp Pro Gln Arg Phe Pro His			
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115	120	125
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155	160		

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175			

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Asp Trp Thr Ser Phe Asn Gln Glu Arg Ile Val Asp Val Ala Gly Pro
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Gly Gly Trp Asn Asp Pro Asp Met Leu Val Ile Gly Asn Phe Gly Leu
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Ser Trp Asn Gln Gln Val Thr Gln Met Ala Leu Trp Ala Ile Met Ala
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Ala Pro Leu Phe Met Ser Asn Asp Leu Arg His Ile Ser Pro Gln Ala
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Lys Ala Leu Leu Gln Asp Lys Asp Val Ile Ala Ile Asn Gln Asp Pro
 305 310 315 320

Leu Gly Lys Gln Gly Tyr Gln Leu Arg Gln Gly Asp Asn Phe Glu Val
 325 330 335

Trp Glu Arg Pro Leu Ser Gly Leu Ala Trp Ala Val Ala Met Ile Asn
 340 345 350

Arg Gln Glu Ile Gly Gly Pro Arg Ser Tyr Thr Ile Ala Val Ala Ser
 355 360 365

Leu Gly Lys Gly Val Ala Cys Asn Pro Ala Cys Phe Ile Thr Gln Leu
 370 375 380

Leu Pro Val Lys Arg Lys Leu Gly Phe Tyr Glu Trp Thr Ser Arg Leu
 385 390 395 400

Arg Ser His Ile Asn Pro Thr Gly Thr Val Leu Leu Gln Leu Glu Asn
 405 410 415

Thr Met Gln Met Ser Leu Lys Asp Leu Ser Glu Lys Asp Glu Leu
 420 425 430

<210> 13

<211> 1278

<212> DNA

<213> Artificial sequence

<220>

<223> Optimized coding sequence of plant derived Alpha GAL II and Chitinase signal peptide fusion protein

<400> 13

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gaattccttg ataatggact tgctaggact ccaactatgg gatggcttca ttggaaacgt	120
ttcatgtgca atctcgattt tcaagagaa ccagattctt gcatttccga gaagctttc	180
atggaaatgg ctgagcttat ggttctgag ggatggagg atgctggata tgagtacctc	240
tgcattcgatg attgctggat ggctccacaa agagattctg agggaaaggct tcaagctgat	300
ccacaaaggt tcccacatgg aattaggcaa ctgcctaact acgttcaact taagggactt	360
aagcttggaa tctacgctga tgtggaaac aagacttgtg ctggatttcc aggatcttcc	420
ggttactacg atatcgatgc tcagactttt gctgattggg gagtggatct tcttaagtcc	480
gatggatgct actgtgattt tcttggaaac ctgcgtatg gttataagca catgtctctc	540
gtcttaata gaactggacg ttccattgtg tattcttgcg agtggccact ttacatgtgg	600
ccattccaga agccaaacta cactgagatt aggcaataact gcaaccattt gaggaaacttc	660
gtgatattt atgattccgt gaagtctatc aagtccatcc ttgattggac ttcatcaat	720
caagaacgta ttgtggatgt tgctggacct ggtggatgaa atgatccaga tatgctcgat	780
atggaaact ttggactttc ttggaaaccag caagttactc aaatggctct ctggctatt	840
atggctgctc cactcttcat gtctaacatc cttaggcaca tttctccaca agctaaggct	900
ttgctccagg ataaggatgt gattgtatc aaccaggatc cacttggaaa gcaaggatac	960
caacttaggc agggtgataa ttttgggtt tgggagggc cactttctgg acttgcttgg	1020
gctgttgcta tgattaacag gcaagagatt ggaggacaa ggttttacac tattgctgtg	1080
gcttctttt gaaagggtgt tgctttaat ccagcttgc tcattactca gcttttgc	1140
gtgaagagga agcttggatt ttaccagtgg acttcttaggc tttagtccaca cattaaccca	1200
actggaaactg tgcttcttca gctcgagaac actatgcaga tgtctttaa ggatttgctc	1260
tccgagaagg atgagctt	1278

<210> 14

<211> 22

<212> PRT

<213> Artificial sequence

<220>

<223> Basic endochitinase B ER targeting signal peptide amino acid sequence

<400> 14

Met	Lys	Thr	Asn	Leu	Phe	Leu	Phe	Leu	Ile	Phe	Ser	Leu	Leu	Leu	Ser
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Leu	Ser	Ser	Ala	Glu	Phe
				20	

<210> 15

<211> 66

<212> DNA

<213> Artificial sequence

<220>

<223> Basic endochitinase B ER targeting signal peptide coding sequence

<400> 15

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gaattc

60

<210> 16

<211> 399

<212> PRT

<213> Artificial sequence

<220>

<223> Recombinant mature Alpah-Gal protein sequence with N' terminal G

<400> 16

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Trp Glu Arg Phe Met Cys Asn Leu Asp Cys Gln Glu Glu Pro Asp Ser
20 25 30

Cys Ile Ser Glu Lys Leu Phe Met Glu Met Ala Glu Leu Met Val Ser
 35 40 45

Glu Gly Trp Lys Asp Ala Gly Tyr Glu Tyr Leu Cys Ile Asp Asp Cys
50 55 60

Trp Met Ala Pro Gln Arg Asp Ser Glu Gly Arg Leu Gln Ala Asp Pro
65 70 75 80

Gln Arg Phe Pro His Gly Ile Arg Gln Leu Ala Asn Tyr Val His Ser
85 90 95

Lys Gly Leu Lys Leu Gly Ile Tyr Ala Asp Val Gly Asn Lys Thr Cys
100 105 110

Aia City The Rio City Set The City Tyr Tyr Asp The Asp Aia City Tyr
115 120 125

130 135 140

145 150 155 160

180 185 190

Cys Asn His Trp Arg Asn Phe Ala Asp Ile Asp Asp Ser Trp Lys Ser
 195 200 205

Ile Lys Ser Ile Leu Asp Trp Thr Ser Phe Asn Gln Glu Arg Ile Val
 210 215 220

Asp Val Ala Gly Pro Gly Gly Trp Asn Asp Pro Asp Met Leu Val Ile
 225 230 235 240

Gly Asn Phe Gly Leu Ser Trp Asn Gln Gln Val Thr Gln Met Ala Leu
 245 250 255

Trp Ala Ile Met Ala Ala Pro Leu Phe Met Ser Asn Asp Leu Arg His
 260 265 270

Ile Ser Pro Gln Ala Lys Ala Leu Leu Gln Asp Lys Asp Val Ile Ala
 275 280 285

Ile Asn Gln Asp Pro Leu Gly Lys Gln Gly Tyr Gln Leu Arg Gln Gly
 290 295 300

Asp Asn Phe Glu Val Trp Glu Arg Pro Leu Ser Gly Leu Ala Trp Ala
 305 310 315 320

Val Ala Met Ile Asn Arg Gln Glu Ile Gly Gly Pro Arg Ser Tyr Thr
 325 330 335

Ile Ala Val Ala Ser Leu Gly Lys Gly Val Ala Cys Asn Pro Ala Cys
 340 345 350

Phe Ile Thr Gln Leu Leu Pro Val Lys Arg Lys Leu Gly Phe Tyr Glu
 355 360 365

Trp Thr Ser Arg Leu Arg Ser His Ile Asn Pro Thr Gly Thr Val Leu
 370 375 380

Leu Gln Leu Glu Asn Thr Met Gln Met Ser Leu Lys Asp Leu Leu
 385 390 395

<210> 17

<211> 4

<212> PRT

<213> Artificial sequence

<220>

<223> Endoplasmic reticulum retention signal peptide

<400> 17

Lys Asp Glu Leu
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<210> 18

<211> 1194

<212> DNA

<213> Artificial sequence

<220>

<223> Optimized coding sequence for mature Alpah-Gal without ABPI or SEKDEL signal peptides

<400> 18

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atggctgagc	ttatggtttc	tgagggatgg	aaggatgctg	gatatgagta	cctctgcatac	180
gatgattgct	ggatggctcc	acaagagat	tctgagggaa	ggcttcaagc	tgatccacaa	240
aggttccac	atggaattag	gcaactcgct	aactacgttc	actctaaggg	acttaagctt	300
ggaatctacg	ctgatgtggg	aaacaagact	tgtgctggat	ttccaggatc	tttcggttac	360
tacgatatacg	atgctcagac	ttttgctgat	tggggagtgg	atcttcttaa	gttcgatgga	420
tgctactgtg	attctcttga	gaacactcgct	gatggttata	agcacatgtc	tctcgctctt	480
aatagaactg	gacgttccat	tgtgtattct	tgcgagtggc	cactttacat	gtggccattc	540
cagaagccaa	actacactga	gattaggcaa	tactgcaacc	attggaggaa	cttcgctgat	600
attgatgatt	cctggaagtc	tatcaagtcc	atccttgatt	ggacttcatt	caatcaagaa	660
cgtattgtgg	atgttgcgtgg	acctgggtgga	tggaatgatc	cagatatgct	cgtgattggaa	720
aactttggac	tttcttgaa	ccagcaagtt	actcaaatgg	ctctctggc	tattatggct	780
gctccactct	tcatgtctaa	cgatcttagg	cacatttctc	cacaagctaa	ggctttgctc	840
caggataagg	atgtgattgc	tatcaaccag	gatccacttg	gaaagcaagg	ataccaactt	900
aggcagggtg	ataattttga	ggttgggag	aggccacttt	ctggacttgc	ttgggctgtt	960
gctatgatta	acaggcaaga	gattggagga	ccaaggcttt	acactattgc	tgtggcttct	1020
cttggaaagg	gtgttgcttg	taatccagct	tgcttcattt	ctcagctttt	gccagtgaag	1080
aggaagcttg	gattttacga	gtggacttct	aggcttaggt	cacacattaa	cccaactggaa	1140
actgtgcttc	ttcagctcga	gaacactatg	cagatgtctc	ttaaggattt	gctc	1194

<210> 19

<211> 18

<212> DNA

<213> Artificial sequence

<220>

<223> Coding sequence for SEKDEL ER retention signal

<400> 19

tccgagaagg atgagctt 18

<210> 20

<211> 404

<212> PRT

<213> Artificial sequence

<220>

<223> Recombinant A-Gal mature protein fused to SEKDEL without N' terminal G

<400> 20

Leu	Asp	Asn	Gly	Leu	Ala	Arg	Thr	Pro	Thr	Met	Gly	Trp	Leu	His	Trp
1				5				10					15		

Glu	Arg	Phe	Met	Cys	Asn	Leu	Asp	Cys	Gln	Glu	Glu	Pro	Asp	Ser	Cys
				20				25					30		

Ile	Ser	Glu	Lys	Leu	Phe	Met	Glu	Met	Ala	Glu	Leu	Met	Val	Ser	Glu
				35				40				45			

Gly	Trp	Lys	Asp	Ala	Gly	Tyr	Glu	Tyr	Leu	Cys	Ile	Asp	Asp	Cys	Trp
				50				55			60				

Met	Ala	Pro	Gln	Arg	Asp	Ser	Glu	Gly	Arg	Leu	Gln	Ala	Asp	Pro	Gln
65				70				75				80			

Arg	Phe	Pro	His	Gly	Ile	Arg	Gln	Leu	Ala	Asn	Tyr	Val	His	Ser	Lys
				85				90				95			

Gly	Leu	Lys	Leu	Gly	Ile	Tyr	Ala	Asp	Val	Gly	Asn	Lys	Thr	Cys	Ala
				100				105				110			

Gly	Phe	Pro	Gly	Ser	Phe	Gly	Tyr	Tyr	Asp	Ile	Asp	Ala	Gln	Thr	Phe
				115				120			125				

Ala	Asp	Trp	Gly	Val	Asp	Leu	Leu	Lys	Phe	Asp	Gly	Cys	Tyr	Cys	Asp
				130				135			140				

Ser	Leu	Glu	Asn	Leu	Ala	Asp	Gly	Tyr	Lys	His	Met	Ser	Leu	Ala	Leu
145					150				155			160			

Asn	Arg	Thr	Gly	Arg	Ser	Ile	Val	Tyr	Ser	Cys	Glu	Trp	Pro	Leu	Tyr
					165			170				175			

Met	Trp	Pro	Phe	Gln	Lys	Pro	Asn	Tyr	Thr	Glu	Ile	Arg	Gln	Tyr	Cys
				180				185			190				

Asn	His	Trp	Arg	Asn	Phe	Ala	Asp	Ile	Asp	Asp	Ser	Trp	Lys	Ser	Ile
				195				200			205				

Lys	Ser	Ile	Leu	Asp	Trp	Thr	Ser	Phe	Asn	Gln	Glu	Arg	Ile	Val	Asp
				210				215			220				

Val	Ala	Gly	Pro	Gly	Gly	Trp	Asn	Asp	Pro	Asp	Met	Leu	Val	Ile	Gly
225					230				235			240			

Asn	Phe	Gly	Leu	Ser	Trp	Asn	Gln	Gln	Val	Thr	Gln	Met	Ala	Leu	Trp
					245			250			255				

Ala	Ile	Met	Ala	Ala	Pro	Leu	Phe	Met	Ser	Asn	Asp	Leu	Arg	His	Ile
					260			265			270				

Ser Pro Gln Ala Lys Ala Leu Leu Gln Asp Lys Asp Val Ile Ala Ile
 275 280 285

Asn Gln Asp Pro Leu Gly Lys Gln Gly Tyr Gln Leu Arg Gln Gly Asp
 290 295 300

Asn Phe Glu Val Trp Glu Arg Pro Leu Ser Gly Leu Ala Trp Ala Val
 305 310 315 320

Ala Met Ile Asn Arg Gln Glu Ile Gly Gly Pro Arg Ser Tyr Thr Ile
 325 330 335

Ala Val Ala Ser Leu Gly Lys Gly Val Ala Cys Asn Pro Ala Cys Phe
 340 345 350

Ile Thr Gln Leu Leu Pro Val Lys Arg Lys Leu Gly Phe Tyr Glu Trp
 355 360 365

Thr Ser Arg Leu Arg Ser His Ile Asn Pro Thr Gly Thr Val Leu Leu
 370 375 380

Gln Leu Glu Asn Thr Met Gln Met Ser Leu Lys Asp Leu Leu Ser Glu
 385 390 395 400

Lys Asp Glu Leu

<210> 21

<211> 405

<212> PRT

<213> Artificial sequence

<220>

<223> Recombinant A-Gal mature protein with N' terminal G and fused to SEKDEL

<400> 21

Gly Leu Asp Asn Gly Leu Ala Arg Thr Pro Thr Met Gly Trp Leu His
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Trp Glu Arg Phe Met Cys Asn Leu Asp Cys Gln Glu Glu Pro Asp Ser
 20 25 30

Cys Ile Ser Glu Lys Leu Phe Met Glu Met Ala Glu Leu Met Val Ser
 35 40 45

Glu Gly Trp Lys Asp Ala Gly Tyr Glu Tyr Leu Cys Ile Asp Asp Cys
 50 55 60

Trp Met Ala Pro Gln Arg Asp Ser Glu Gly Arg Leu Gln Ala Asp Pro
 65 70 75 80

Gln Arg Phe Pro His Gly Ile Arg Gln Leu Ala Asn Tyr Val His Ser
 85 90 95

Lys Gly Leu Lys Leu Gly Ile Tyr Ala Asp Val Gly Asn Lys Thr Cys

- - - 100 - - - 105 - - - 110 - - -

Ala Gly Phe Pro Gly Ser Phe Gly Tyr Tyr Asp Ile Asp Ala Gln Thr
 115 120 125

Phe Ala Asp Trp Gly Val Asp Leu Leu Lys Phe Asp Gly Cys Tyr Cys
 130 135 140

Asp Ser Leu Glu Asn Leu Ala Asp Gly Tyr Lys His Met Ser Leu Ala
 145 150 155 160

Leu Asn Arg Thr Gly Arg Ser Ile Val Tyr Ser Cys Glu Trp Pro Leu
 165 170 175

Tyr Met Trp Pro Phe Gln Lys Pro Asn Tyr Thr Glu Ile Arg Gln Tyr
 180 185 190

Cys Asn His Trp Arg Asn Phe Ala Asp Ile Asp Asp Ser Trp Lys Ser
 195 200 205

Ile Lys Ser Ile Leu Asp Trp Thr Ser Phe Asn Gln Glu Arg Ile Val
 210 215 220

Asp Val Ala Gly Pro Gly Trp Asn Asp Pro Asp Met Leu Val Ile
 225 230 235 240

Gly Asn Phe Gly Leu Ser Trp Asn Gln Gln Val Thr Gln Met Ala Leu
 245 250 255

Trp Ala Ile Met Ala Ala Pro Leu Phe Met Ser Asn Asp Leu Arg His
 260 265 270

Ile Ser Pro Gln Ala Lys Ala Leu Leu Gln Asp Lys Asp Val Ile Ala
 275 280 285

Ile Asn Gln Asp Pro Leu Gly Lys Gln Gly Tyr Gln Leu Arg Gln Gly
 290 295 300

Asp Asn Phe Glu Val Trp Glu Arg Pro Leu Ser Gly Leu Ala Trp Ala
 305 310 315 320

Val Ala Met Ile Asn Arg Gln Glu Ile Gly Gly Pro Arg Ser Tyr Thr
 325 330 335

Ile Ala Val Ala Ser Leu Gly Lys Gly Val Ala Cys Asn Pro Ala Cys
 340 345 350

Phe Ile Thr Gln Leu Leu Pro Val Lys Arg Lys Leu Gly Phe Tyr Glu
 355 360 365

Trp Thr Ser Arg Leu Arg Ser His Ile Asn Pro Thr Gly Thr Val Leu
 370 375 380

Leu Gln Leu Glu Asn Thr Met Gln Met Ser Leu Lys Asp Leu Leu Ser
 385 390 395 400

Glu Lys Asp Glu Leu

405

<210> 22

<211> 1212

<212> DNA

<213> Artificial sequence

<220>

<223> Optimized coding sequence for mature A-Gal protein fused to SEKDEL without ABPI

<400> 22

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tgcaatctcg	attgtcaaga	ggaaccagat	tcttgcattt	ccgagaagct	tttcatggaa	120
atggctgagc	ttatggttc	tgagggatgg	aaggatgctg	gatatgagta	cctctgcattc	180
gatgattgct	ggatggctcc	acaaagagat	tctgagggaa	ggcttcaagc	tgtccacaa	240
aggttcccac	atggaattag	gcaactcgct	aactacgttc	actctaaggg	acttaagctt	300
ggaatctacg	ctgatgtggg	aaacaagact	tgtgctggat	ttccaggatc	tttcggttac	360
tacgatatacg	atgctcagac	ttttgctgat	tggggagtgg	atcttcttaa	gttcgatgga	420
tgctactgtg	attctcttga	gaacctcgct	gatggttata	agcacatgtc	tctcgcttt	480
aatagaactg	gacgttccat	tgtgtattct	tgcgagtggc	cactttacat	gtggccattc	540
cagaagccaa	actacactga	gattaggcaa	tactgcaacc	attggagggaa	cttcgctgat	600
attgatgatt	cctgaaagtgc	tatcaagtcc	atccttgatt	ggacttcatt	caatcaagaa	660
cgtattgtgg	atgttgctgg	acctgggtgg	tggaatgatc	cagatatgct	cgtgattggaa	720
aactttggac	tttcttgaaa	ccagcaagtt	actcaaatgg	ctctctgggc	tattatggct	780
gctccactct	tcatgtctaa	cgatcttagg	cacatttctc	cacaagctaa	ggctttgctc	840
caggataagg	atgtgattgc	tatcaaccag	gatccacttg	gaaagcaagg	ataccaaactt	900
aggcagggtg	ataattttga	ggttgggag	aggccacttt	ctggacttgc	ttgggctgtt	960
gctatgatta	acaggcaaga	gattggagga	ccaaggcttt	acactattgc	tgtggcttct	1020
cttggaaagg	gtgttgcttg	taatccagct	tgcttcatta	ctcagctttt	gccagtgaag	1080
aggaagcttgc	gatttacga	gtggacttct	aggcttaggt	cacacattaa	cccaactggaa	1140
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aaggatgagc	tt					1212

<210> 23

<211> 1215

<212> DNA

<213> Artificial sequence

<220>

<223> Optimized coding sequence for mature A-Gal protein with N' terminal G and SEKDEL

<400> 23

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------------	------------	------------	-----------	------------	------------	----

atgtgcaatc tcgattgtca agaggaacca gattcttgc a tttccgagaa gcttttcatg	120
gaaatggctg agcttatgg t tctgaggga t ggaaggatg ctggatatga gtacctctgc	180
atcgatgatt gctggatggc tccacaaaga gattctgagg gaaggcttca agctgatcca	240
caaaggttcc cacatggat taggcaactc gctaactacg ttcactctaa gggacttaag	300
cttggaatct acgctgatgt gggaaacaag acttgtgctg gatttccagg atcttcggt	360
tactacgata tcgatgctca gactttgct gattggggag tggatcttct taagttcgat	420
ggatgctact gtgattctct tgagaacctc gctgatggtt ataagcacat gtctctcgct	480
cttaatagaa ctggacgttc cattgtgtat tcttgcgagt ggccacttta catgtggcca	540
ttccagaagc caaactacac tgagattagg caatactgca accattggag gaacttcgct	600
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gaacgtattt gggatgttgc tggacctggt gatggaaatg atccagatat gctcgtgatt	720
ggaaactttt gactttctt g aaccagcaa gttactcaaa tggctctctg ggctattatg	780
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gttgctatga ttaacaggca agagattgga gaccaaggt cttacactat tgctgtggct	1020
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aagaggaagc ttggattttt c gaggact tctaggctt ggtcacacat taacccaact	1140
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gagaaggatg agctt	1215

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ALPHA-GALACTOSIDASE-SAMMENSÆTNINGER
PATENTKRAV

1. Humant α -galactosidase-protein omfattende en glycin som den N-terminale remanens, hvor det pågældende humane α -galactosidase-protein fusioneres translationelt ved C-terminalen til et endoplasmatisk reticulumretentionssignalpeptid, og hvor det humane α -galactosidase-protein kan opnås ved at udtrykke proteinet i en plantecelle, der betår af en nukleinsyrekonstruktion omfattende en nukleinsyresekvens, der koder et humant α -galactosidase-protein, der fusioneres translationelt ved N-terminalen til et *Arabidopsis* ABPI-endoplasmatiske reticulummålretningssignalpeptid og fusioneres translationelt ved C-terminalen til et endoplasmatisk reticulumretentionssignalpeptid.
2. Humant α -galactosidase-protein ifølge krav 1, der består af den humane α -galactosidase-protein-aminosyresekvens som angivet i SEQ ID NO: 21.
3. Humant α -galactosidase-protein ifølge krav 1 eller 2, hvor det pågældende endoplasmatiske reticulumretentionssignal har en aminosyresekvens som angivet i SEQ ID NO: 6.
4. Humant α -galactosidase-protein ifølge et hvilket som helst af kravene 1-3, hvor den pågældende nukleinsyresekvens, der koder det pågældende humane α -galactosidase-protein, der fusioneres translationelt ved N-terminalen til et *Arabidopsis* ABPI-endoplasmatiske reticulummålretningssignalpeptid og fusioneres translationelt ved C-terminalen til et endoplasmatisk reticulumretentionssignalpeptid, kodonoptimeres til ekspression i tobaksplanteceller.
5. Humant α -galactosidase-protein ifølge et hvilket som helst af kravene 1-3, hvor den pågældende nukleinsyresekvens, der koder det pågældende humane α -galactosidase-protein, som fusioneres translationelt ved N-terminalen til et *Arabidopsis* ABPI-endoplasmatiske reticulummålretningssignalpeptid og fusioneres translationelt ved C-terminalen til et endoplasmatisk reticulumretentionssignalpeptid, omfatter SEQ ID NO: 2.
6. Humant α -galactosidase-protein ifølge et hvilket som helst af kravene 1-3, hvor

det pågældende humane α -galactosidase-protein er et oprenset, humant α -galactosidase-protein.

7. Humant α -galactosidase-protein ifølge et hvilket som helst af kravene 1-6, hvor det pågældende humane α -galactosidase-protein er katalytisk aktivt som bestemt ved *p*-nitrophenylalpha-*D*- galactopyranosid-analyse.

8. Humane α -galactosidase-proteiner ifølge et hvilket som helst af kravene 1-6, hvor mindst 0,5 % af den pågældende flerhed af humane α -galactosidase-proteiner har en glycanstruktur, der omfatter ni mannosester, hvor tre er eksponerede mannosester.

9. Flerhed af humane α -galactosidase-proteiner ifølge krav 8, hvor de fremherskende glycanstrukturer i den pågældende flerhed af humane α -galactosidase-proteiner er mannose 4- β -(1,2) xylose (M4X); mannose 3- β -(1,2) xylose-a-(1,3) fucose [Fc (3) M3X] og mannose 8 (M8) samt en farmaceutisk acceptabel bærer.

10. Isoleret plantecelle omfattende det humane α -galactosidase-protein ifølge et hvilket som helst af kravene 1-7 eller flerheden af humane α -galactosidase-proteiner ifølge krav 8 eller 9.

11. Isoleret plantecelle ifølge krav 10, hvor den pågældende plantecelle er en celle i en tobakscellelinje.

12. Isoleret plantecelle ifølge krav 10, hvor den pågældende plantecelle er en BY-2-celle.

13. Farmaceutisk sammensætning, der omfatter, som en aktiv ingrediens, det humane α -galactosidase-protein ifølge til et hvilket som helst af kravene 1-7 eller flerheden af humane α -galactosidase-proteiner ifølge krav 7 eller krav 8 og en farmaceutisk acceptabel bærer.

14. Farmaceutisk sammensætning, der omfatter, som en aktiv ingrediens, plantecellen ifølge et hvilket som helst af kravene 10-12 og en farmaceutisk acceptabel bærer.

15. Humant α -galactosidase-protein ifølge et hvilket som helst af kravene 1-7, flerheden af humane α -galactosidase-proteiner følge krav 7 eller krav 8, den isolerede plantecelle ifølge et hvilket som helst af kravene 10-12 eller den farmaceutiske sammensætning ifølge krav 13 eller 14 til anvendelse i behandling af Fabrys sygdom hos et individ, der har behov for det.

DRAWINGS

[REDACTED] LDNGIARTPTIMGWLHWE
[REDACTED] LDNGIARTPTIMGWLHWE
1 50

RFMCNLDCQEEPDS CISEKLFMEMAELM/SEGWKDAGYEYLCIDDCWMA P
RFMCNLDCQEEPDS CISEKLFMEMAELM/SEGWKDAGYEYLCIDDCWMA P
51 100

QRDSEGRIQADPQRFPHGIQLANYVHSKGLKIGIYADVGNKTCAGFPG S
QRDSEGRIQADPQRFPHGIQLANYVHSKGLKIGIYADVGNKTCAGFPG S
101 150

FGYYDIDAQTFADNGVDLLKFDGCYCDSLENLADGYKHMSLAINRTGRS I
FGYYDIDAQTFADNGVDLLKFDGCYCDSLENLADGYKHMSLAINRTGRS I
151 200

VYSCEWPLYMWPFQKPNYTEIRQYCNHWRNFADI DDSWKSIIKSILDWTS F
VYSCEWPLYMWPFQKPNYTEIRQYCNHWRNFADI DDSWKSIIKSILDWTS F
201 250

NQERIVDVAGPGGWNDPDMLVIGNFGLSWNQQVTQMAIWAIMAAPLFMSN
NQERIVDVAGPGGWNDPDMLVIGNFGLSWNQQVTQMAIWAIMAAPLFMSN
251 300

DLRHISPQAKALIQDKDVIAINQDPLGKQGYQIRQGDNFEVWERPLSGLA
DLRHISPQAKALIQDKDVIAINQDPLGKQGYQIRQGDNFEVWERPLSGLA
301 350

WAVAMINRQEIGGPRSYTIAVASLGKGVACNPACFITQLLPVKRKLGFYE
WAVAMINRQEIGGPRSYTIAVASLGKGVACNPACFITQLLPVKRKLGFYE
351 400

WTSLRLSHINPTGTIVLLQLENTMQMSLKDLL
WTSLRLSHINPTGTIVLLQLENTMQMSLKDLLSEKDEL
401 427

FIG. 1

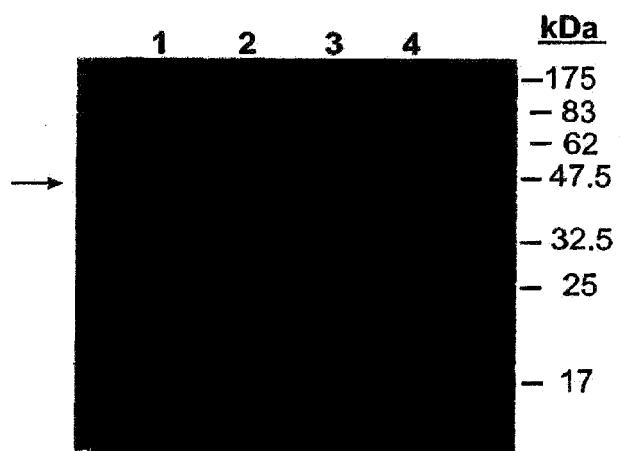


FIG. 2

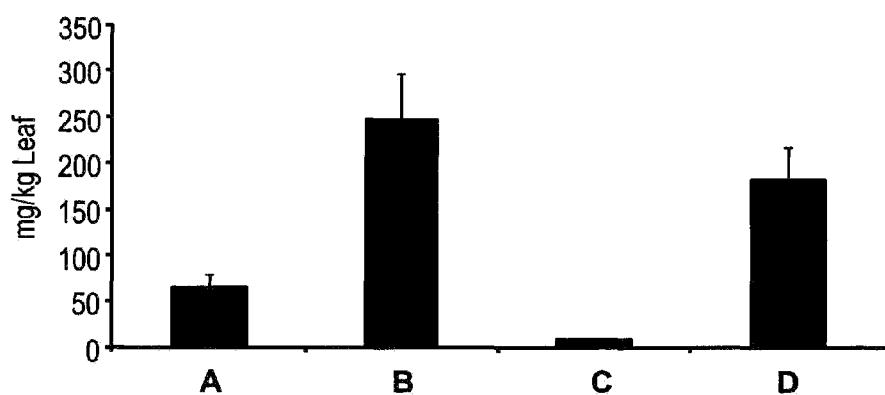


FIG. 3

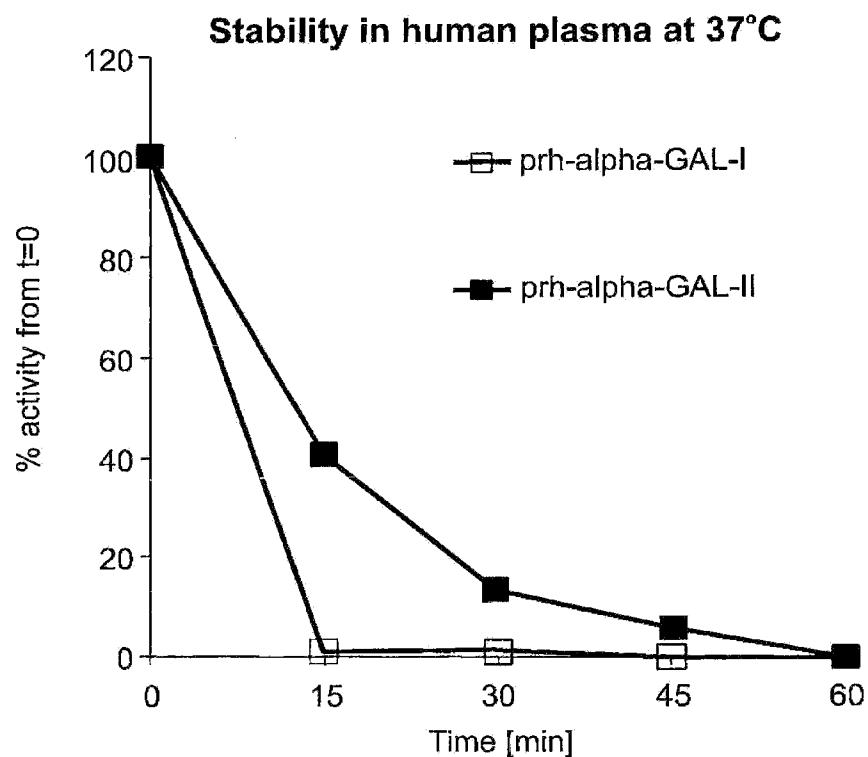


FIG. 4

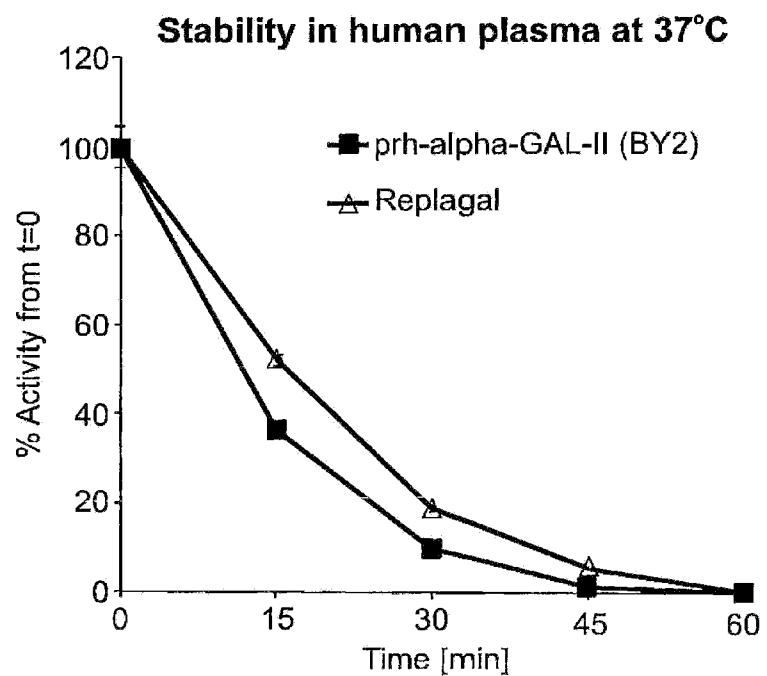


FIG. 5

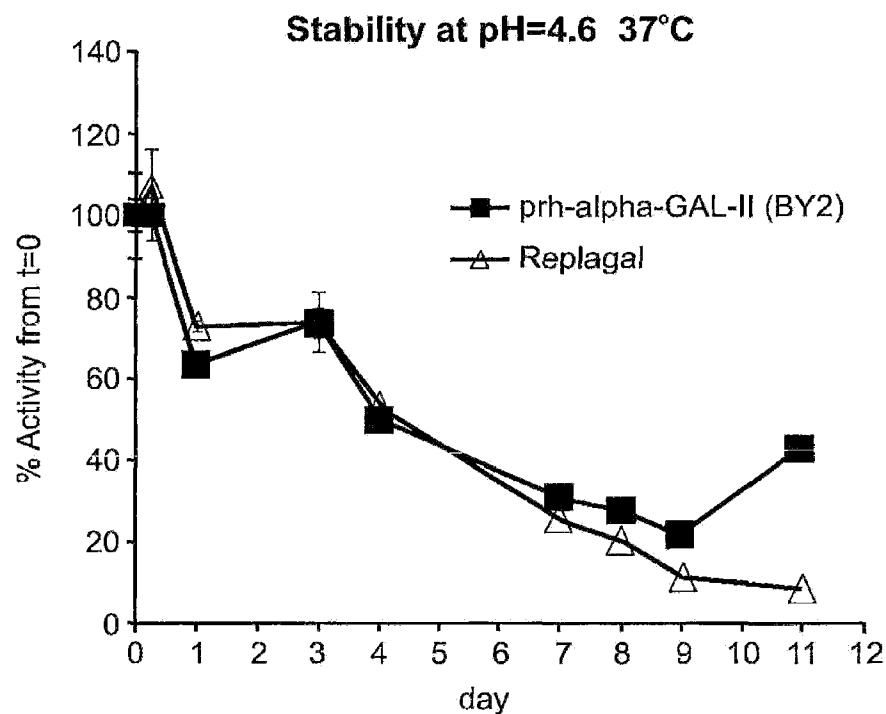


FIG. 6

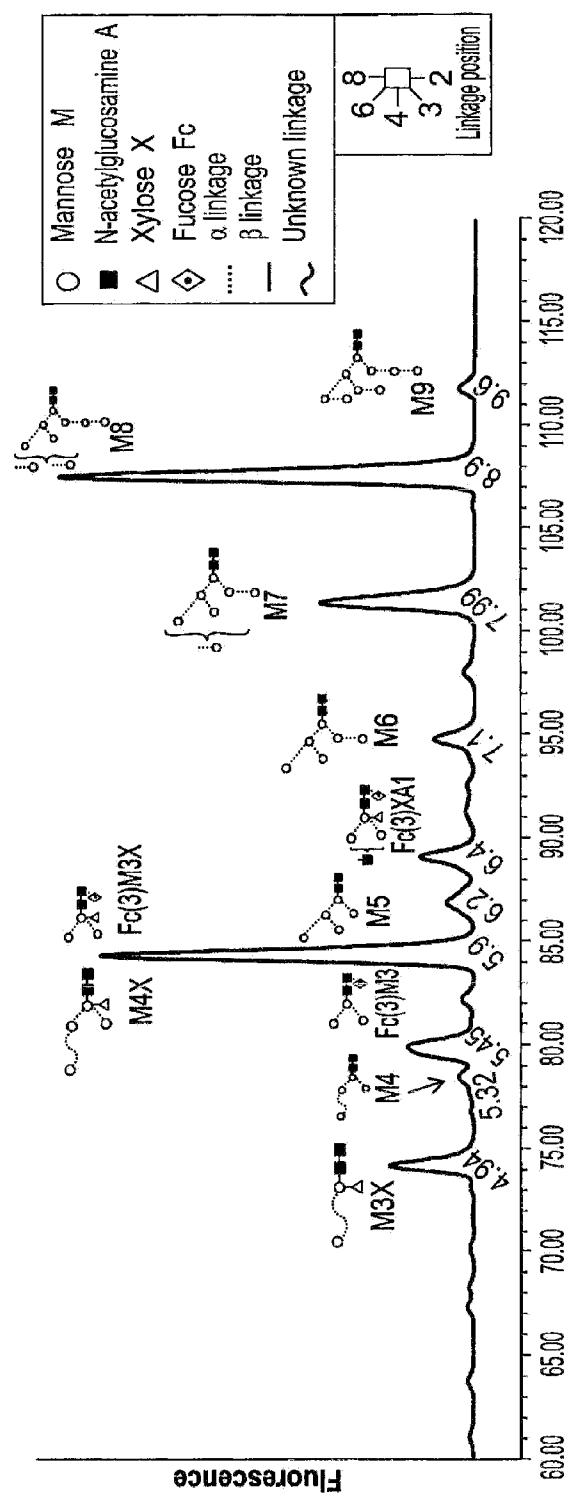


FIG. 7

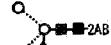
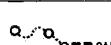
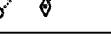
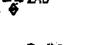
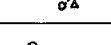
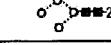
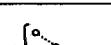
	Name	Structure	Retention Time	GU	A-GAL batch A	A-GAL batch B
1	FcM2X		71.83	4.76	NA	NA
2	M3X		74.32	4.9	6.41	6.51
3	M4		78.5	5.31	1.16	1.19
4	FcM3		79.86	5.32	7.17	6.72
5	FcM3X or M4X		84.55	5.9	26.94	27.59
6	M5		87.126	6.19	3.24	2.44
8	M6		95.083	7.06	3.41	3.34
9	M7		101.81	7.88	14.04	14.27
10	M8		108.05	8.83	31.64	32.12
11	M9		112.347	9.48	1.2	1.3

FIG. 8