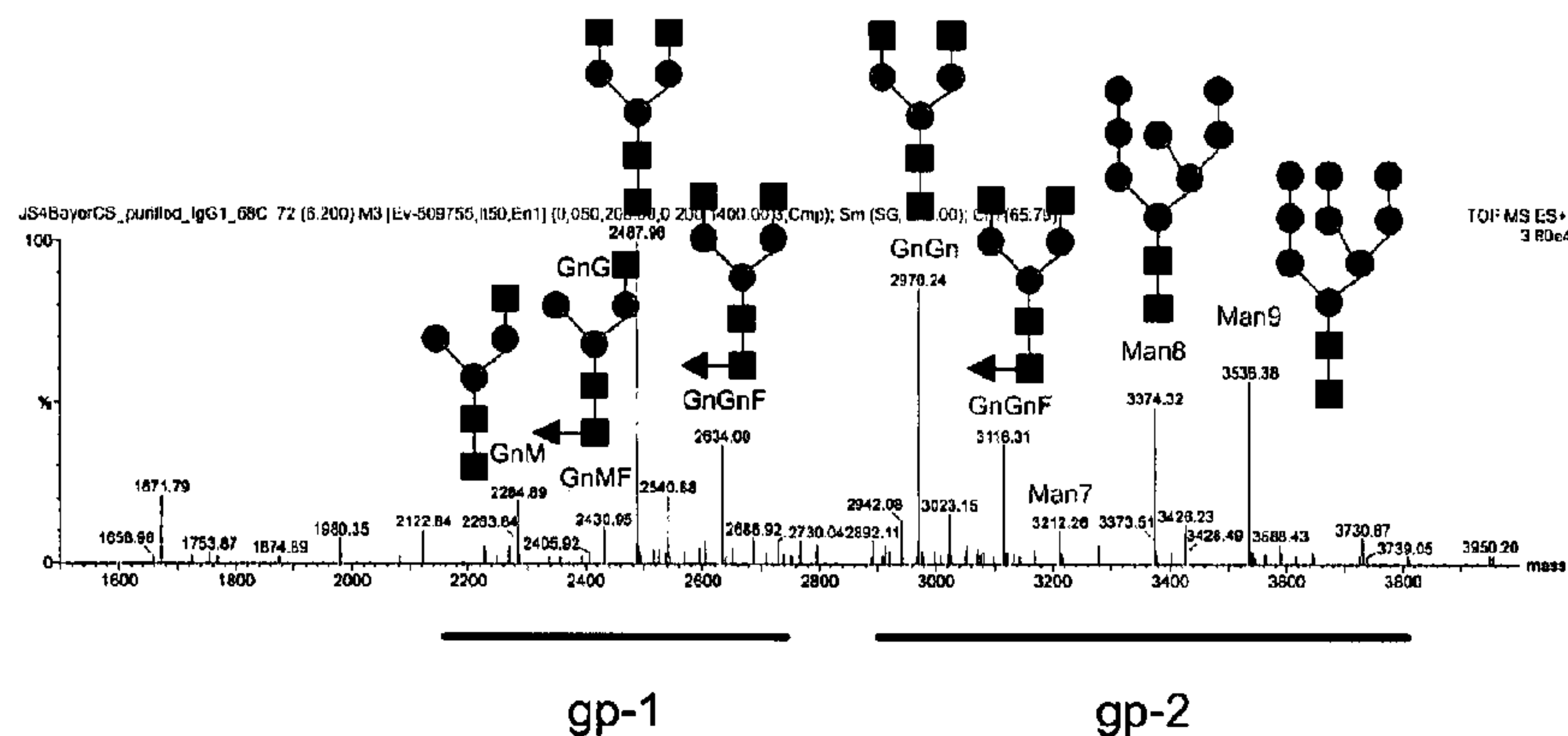




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(54) Title: NICOTIANA BENTHAMIANA PLANTS DEFICIENT IN XYLOSYLTRANSFERASE ACTIVITY



(57) **Abrégé/Abstract:**

The invention provides *Nicotiana benthamiana* mutant plants which are incapable of forming xylosyl-structures on glycoproteins. In addition, the invention provides methods for the production of heterologous glycoproteins in said mutant plants.

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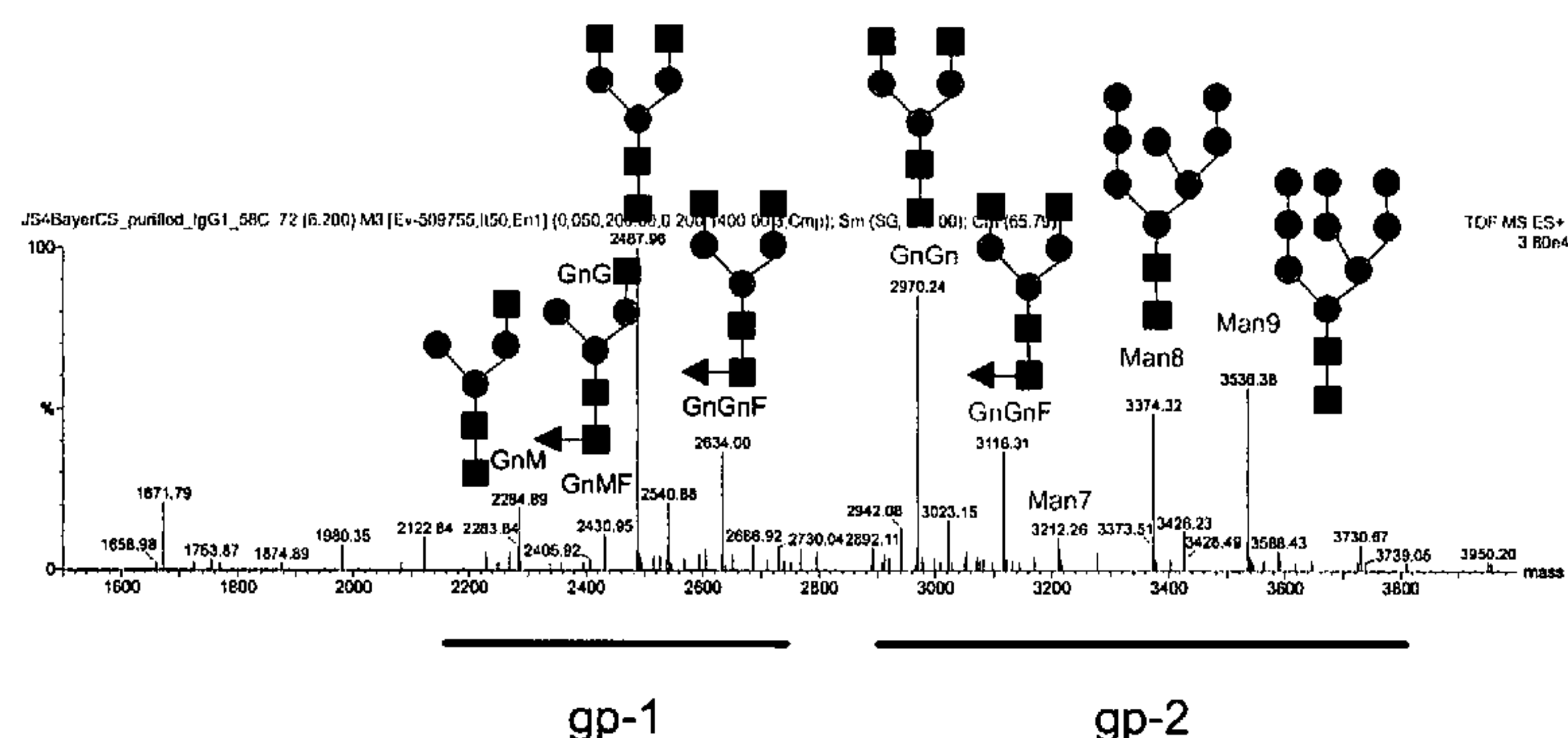


Figure 4

(57) Abstract: The invention provides *Nicotiana benthamiana* mutant plants which are incapable of forming xylosyl-structures on glycoproteins. In addition, the invention provides methods for the production of heterologous glycoproteins in said mutant plants.

Nicotiana benthamiana* plants deficient in xylosyltransferase activity*Field of the invention**

5 The current invention relates to the field of molecular farming, i.e. the use of plants and plant cells as bioreactors to produce biopharmaceuticals, particularly proteins with pharmaceutical interest such as therapeutic proteins, which have an N-glycosylation pattern that resembles mammalian glycosylation. The invention relates to plants of the genus *Nicotiana* which are deficient in xylosyltransferase
10 activity, which plants may be applied as host plants or host cells to produce heterologous glycoproteins.

Background

15 Glycosylation is the covalent linkage of an oligosaccharide chain to a protein resulting in a glycoprotein. In many glycoproteins, the oligosaccharide chain is attached to the amide nitrogen of an asparagine (Asn) residue and leads to N-glycosylation. Glycosylation represents the most widespread post-translational modification found in natural and biopharmaceutical proteins. It is estimated that
20 more than half of the human proteins are glycosylated and their function frequently depends on particular glycoforms (glycans), which can affect their plasma half life, tissue targeting or even their biological activity. Similarly, more than one-third of approved biopharmaceuticals are glycoproteins and both their function and efficiency are affected by the presence and composition of their N-
25 glycans. Leafy crops, such as the tobacco plant *Nicotiana benthamiana*, are an attractive system for the production of therapeutic proteins, as plants are generally considered to have several advantages, including the lack of animal pathogens such as prions and viruses, low cost and the large-scale production of safe and biologically active valuable recombinant proteins, the ease of scale-up,
30 efficient harvesting and storage possibilities. However, N-linked glycans from plants differ from those of mammalian cells. For example in plants, beta-(1,2)-

xylose residues have been shown to be linked to the core Man3GlucNAc2-Asn of glycans, whereas they are not detected on mammalian glycans, where sialic acid residues and terminal beta(1,4)-galactosyl structures occur instead. The unique N-glycans added by plants could impact on both immunogenicity and functional activity of the protein and, consequently, may represent a limitation for plants to be used as a protein production platform. Indeed, the immunogenicity of beta-1,2-xylose residues in mammals has been described in for example Jin et al. (2006) Glycobiology 16: 349- 357.

The enzyme that catalyses the transfer of xylose from UDP-xylose to the core β -linked mannose of protein-bound N-glycans is beta-1,2-xylosyltransferase ("XylT", EC 2.4.2.38). The beta-1,2-xylosyltransferase is an enzyme unique to plants and some non-vertebrate animal species and does not occur in human beings or in other vertebrates.

WO2007107296 describes the identification and cloning of beta-1,2 xylosyltransferases from the genus *Nicotiana* such as *Nicotiana benthamiana*. Various strategies have been applied to avoid beta-1,2-xylosyl structures on glycoproteins produced by plants. WO2009056155 describes an RNA interference strategy for the generation of *Nicotiana benthamiana* plants which are deficient in the formation of beta-1,2-xylosyl structures as well as devoid of alfa-1,3-fucosyl structures on heterologous glycoproteins.

The cleanest approach for the production of glycoproteins lacking xylosyl-epitopes in *Nicotiana benthamiana* would be the generation of a full knock-out of the beta-1,2-xylosyltransferase gene in this plant. The latter is however not a straight-forward strategy because of the documented presence of at least two beta-1,2-xylosyltransferases (see WO2007107296) and the extremely low efficiency of homologous recombination in plants. Another strategy would be the generation of null mutations in all of the functional alleles of the genes possessing beta-1,2 xylosyltransferase activity in *Nicotiana benthamiana*. Plant populations mutagenized by ethyl methanesulfonate (EMS) have proved

invaluable to plant biologists as a means of dissecting genomic traits. *Nicotiana benthamiana* is however a higher plant and is estimated to contain 30.000 to 50.000. A major obstacle in *Nicotiana benthamiana* genetics is the lack of large mutant populations required for mutant gene identification. Such a useful *N. benthamiana* population would ideally contain at least one mutant allele for every *N. benthamiana* gene. Mutant *N. benthamiana* plants can be produced through the use of DNA damaging agents such as EMS, X-rays, or fast-neutrons. However, no stocks of mutagenized M2 seeds, originating from a large population of M1 plants, are available for screening mutations in candidate genes.

10 The aim of our research was to provide a mutant population of *N. benthamiana*, to screen for null alleles in said population for genes that encode beta-1,2-xylosyltransferase activity with the ultimate goal to evaluate the possibility of obtaining an induced mutant plant completely deficient in the xylosyltransferase activity.

15 Summary of the invention

In work leading up to the present invention, the inventors sought to inactivate by classical mutagenesis the beta-1,2-xylosyltransferase pathway in *Nicotiana benthamiana* which is involved in undesired N-glycosylation hampering the usefulness for the production of heterologous proteins in higher plants. In particular, the inventors have chemically mutagenized a wild type *Nicotiana benthamiana* plant and have identified null alleles of two beta-1,2-xylosyltransferase genes by classical mutagenesis in *Nicotiana benthamiana*. After combining said null alleles in a single plant, it was observed that homozygous double mutant *Nicotiana benthamiana* plant – comprising a

20 homozygous combination of the four null alleles – proved to be viable and revealed no obvious, morphological phenotype under standard growth conditions. Most importantly, the resulting homozygous double mutant *N. benthamiana* plant was devoid of the complete xylosyltransferase pathway because it produced endogenous and heterologous glycoproteins which lacked beta-1,2-xylosyl sugar

25 structures on said glycoproteins. Thus, the homozygous combination of the four

30

null alleles proved to be sufficient for the elimination of the complete beta-1,2-xylosyltransferase activity in *Nicotiana benthamiana*.

It is therefore one object of the invention to provide a beta-1,2-xylosyltransferase null mutant of the plant *Nicotiana benthamiana*, or cells, parts, seed or progeny thereof, reference seed having been deposited on May 21, 2009 at the NCIMB under accession number NCIMB 41622. It is another object to provide a *Nicotiana benthamiana* plant or plant cell which is a beta-1,2-xylosyltransferase null mutant characterized by comprising a combination of homozygous null alleles selected from a null allele from the group consisting of xyltg14-1, xyltg14-2 or xyltg14-3 and a null allele selected from the group consisting of xyltg19-1 or xyltg19-2. It is a further object to provide a plant or plant cell which does not form detectable levels of beta-1,2-xylosyl-sugars on N-glycan structures of glycoproteins produced in said plant.

It is another object to provide a *Nicotiana benthamiana* seed characterized as being homozygous for two null alleles, xyltg14-1 and xyltg19-1, of beta-1,2-xylosyltransferase, having been deposited at the NCIMB on May 21, 2009, under accession number NCIMB 41622.

In a further object a *Nicotiana benthamiana* plant, or a cell, part, seed or progeny thereof, obtained from the reference seed having been deposited at the NCIMB on May 21, 2009, under accession number NCIMB 41622.

In yet a further object the beta-1,2-xylosyltransferase null mutant of the plant or plant cell of *Nicotiana benthamiana* further comprises a silenced alpha-1,3-fucosyltransferase activity.

In yet another further embodiment the beta-1,2-xylosyltransferase null mutant of the plant or plant cell of *Nicotiana benthamiana* that comprises a silenced alpha-1,3-fucosyltransferase activity in addition also comprises a beta-1,4-galactosyltransferase activity.

In yet another further embodiment the beta-1,2-xylosyltransferase null mutant of the plant or plant cell of *Nicotiana benthamiana* that comprises a silenced alpha-1,3-fucosyltransferase activity and a beta-1,4-galactosyltransferase activity further comprises a chimeric gene encoding a heterologous protein.

- 5 In yet a further aspect the *Nicotiana benthamiana* plants described herein before are used for the production of heterologous proteins.

In yet a further aspect a method is provided to produce at least one heterologous protein in plants or plant cells described herein before, comprising the steps of a) providing a plant or plant cell described herein before, with at least one chimeric gene comprising the following
 10 operably linked nucleic acid molecules: i) a plant-expressible promoter, ii) a DNA region encoding a heterologous protein, and iii) a DNA region involved in transcription termination and polyadenylation, and b) cultivating said plant or plant cell and isolating said at least one heterologous protein from said plant or plant cell. In a particular aspect said heterologous protein is an antibody.

- 15 The present invention as claimed relates to:

- a cell of a beta-1,2-xylosyltransferase null mutant of the plant *Nicotiana benthamiana*, or progeny cell thereof, said null mutant being obtained by breeding with a plant grown from reference seed having been deposited on May 21, 2009 at the NCIMB under accession number NCIMB 41622, wherein said cell comprises null alleles xyltg14-1 and xyltg19-1 in
 20 homozygous state; wherein allele xyltg14-1 comprises a C → T mutation corresponding to position 192 in SEQ ID NO: 1, thereby introducing a STOP codon; and allele xyltg19-1 comprises a C → T mutation corresponding to position 139 in SEQ ID NO: 3, thereby introducing a STOP codon;
- a *Nicotiana benthamiana* beta-1,2-xylosyltransferase null mutant plant cell comprising a
 25 combination of homozygous null alleles: a null allele selected from xyltg14-1, xyltg14-2 or xyltg14-3; and a null allele selected from xyltg19-1 or xyltg19-2; wherein allele xyltg14-1 comprises a C → T mutation corresponding to position 192 in SEQ ID NO: 1, thereby introducing a STOP codon; wherein allele xyltg14-2 comprises a G → A mutation corresponding to position 212 in SEQ ID NO: 1, thereby introducing a STOP codon;

wherein allele xyltg14-3 comprises a G → A mutation corresponding to position 329 in SEQ ID NO: 1, thereby introducing a STOP codon; wherein allele xyltg19-1 comprises a C → T mutation corresponding to position 139 in SEQ ID NO: 3, thereby introducing a STOP codon; and wherein allele xyltg19-2 comprises a G → A mutation corresponding to position 183 in SEQ ID NO: 3, thereby introducing a STOP codon;

- a *Nicotiana benthamiana* plant cell, comprising a combination of homozygous null alleles: a null allele selected from xyltg14-1, xyltg14-2 or xyltg14-3; and a null allele selected from xyltg19-1 or xyltg19-2; wherein allele xyltg14-1 comprises a C → T mutation corresponding to position 192 in SEQ ID NO: 1, thereby introducing a STOP codon; wherein allele xyltg14-2 comprises a G → A mutation corresponding to position 212 in SEQ ID NO: 1, thereby introducing a STOP codon; wherein allele xyltg14-3 comprises a G → A mutation corresponding to position 329 in SEQ ID NO: 1, thereby introducing a STOP codon; wherein allele xyltg19-1 comprises a C → T mutation corresponding to position 139 in SEQ ID NO: 3, thereby introducing a STOP codon; and wherein allele xyltg19-2 comprises a G → A mutation corresponding to position 183 in SEQ ID NO: 3, thereby introducing a STOP codon;

- a cell of a *Nicotiana benthamiana* seed, wherein the cell is homozygous for two null alleles, xyltg14-1 and xyltg19-1, of beta-1,2-xylosyltransferase, the seed having been deposited at the NCIMB on May 21, 2009, under accession number NCIMB 41622, wherein allele xyltg14-1 comprises a C → T mutation corresponding to position 192 in SEQ ID NO: 1, thereby introducing a STOP codon; and allele xyltg19-1 comprises a C → T mutation corresponding to position 139 in SEQ ID NO: 3, thereby introducing a STOP codon;

- a *Nicotiana benthamiana* plant cell, or progeny cell obtained from seed deposited at the NCIMB on May 21, 2009, under accession number NCIMB 41622, wherein the plant cell or progeny cell is homozygous for two null alleles, xyltg14-1 and xyltg19-1 of beta-1,2-xylosyltransferase, wherein allele xyltg14-1 comprises a C → T mutation corresponding to position 192 in SEQ ID NO: 1, thereby introducing a STOP codon; and allele xyltg19-1 comprises a C → T mutation corresponding to position 139 in SEQ ID NO: 3, thereby introducing a STOP codon; and

- a method to produce at least one heterologous protein in plants or plant cells, comprising the steps of: a. providing a plant or plant cell with at least one chimeric gene comprising the following operably linked nucleic acid molecules: (a) a plant-expressible promoter, (b) a DNA region encoding a heterologous protein, and (c) a DNA region involved in transcription termination and polyadenylation, and b. cultivating said plant or plant cell and isolating said at least one heterologous protein from said plant or plant cell; wherein said plant or plant cell of item a. is (i) a *Nicotiana benthamiana* beta-1,2-xylosyltransferase null mutant plant or cell or progeny thereof, wherein the null mutant plant or cell is obtained by breeding with a plant grown from reference seed having been deposited on May 21, 2009 at the NCIMB under accession number NCIMB 41622, wherein said null mutant plant or cell or progeny thereof comprises null alleles xyltg14-1 and xyltg19-1 in homozygous state; (ii) a *Nicotiana benthamiana* beta-1,2-xylosyltransferase null mutant plant or plant cell, comprising a combination of homozygous null alleles: a null allele selected from xyltg14-1, xyltg14-2 or xyltg14-3, and a null allele selected from xyltg19-1 or xyltg19-2; (iii) a *Nicotiana benthamiana* plant or cell, comprising a combination of homozygous null alleles: a null allele selected from xyltg14-1, xyltg14-2 or xyltg14-3, and a null allele selected from xyltg19-1 or xyltg19-2; or (iv) a *Nicotiana benthamiana* plant or cell or progeny thereof, wherein the plant or cell is obtained from the seed having been deposited at the NCIMB on May 21, 2009, under accession number NCIMB 41622, said plant or cell or progeny thereof being homozygous for two null alleles, xyltg14-1 and xyltg19-1, of beta-1,2-xylosyltransferase; wherein allele xyltg14-1 comprises a C → T mutation corresponding to position 192 in SEQ ID NO: 1, thereby introducing a STOP codon; wherein allele xyltg14-2 comprises a G → A mutation corresponding to position 212 in SEQ ID NO: 1, thereby introducing a STOP codon; wherein allele xyltg14-3 comprises a G → A mutation corresponding to position 329 in SEQ ID NO: 1, thereby introducing a STOP codon; wherein allele xyltg19-1 comprises a C → T mutation corresponding to position 139 in SEQ ID NO: 3, thereby introducing a STOP codon; wherein allele xyltg19-2 comprises a G → A mutation corresponding to position 183 in SEQ ID NO: 3, thereby introducing a STOP codon.

Figures

Figure 1: Fig. 1 A. Determination of the optimum EMS dose for production of M2 seeds in *N. benthamiana*. Seeds were treated with different concentrations of EMS and the effect on seed survival is shown. Fig. 1 B. Determination of the optimum EMS dose for
5 production of M2 seeds in *N. benthamiana*. Seeds were treated with different concentrations of EMS and the effect on plant fertility was recorded.

Figure 2A and 2B: Summary of the position of SNPs between accessions BENTHAMIANA and NBNPGS2 and of mutant alleles in XylTg14 and XylTg19. The SNP and name of allele or accession are indicated above the sequence. The area searched for EMS mutations
10 and/or SNPs is underlined.

Figure 3: Absence of detectable beta-1,2-xylose sugars on N-glycans of total protein from plants homozygous for XylTg14-1 and XylTg19-1. Left panel: western blot probed with anti-xylose antibody; right panel: western blot probed with anti-fucose antibody. – (negative) control: no protein loaded; + (positive) control: protein from wt BENTHAMIANA accession; Het 2KO: protein from double heterozygous mutant; single KO (14): protein from plant homozygous for XylTg14-1; single KO (19): protein from plant homozygous for XylTg19-1; Double KO: protein from plant homozygous for XylTg14-1 and -19-1. 10 µg total protein was loaded per lane.

Figure 4: N-glycan analysis of heavy chain of a magnICON[®]-expressed IgG1 from a homozygous XylTg14-1 and XylTg19-1 double knock out *N. benthamiana* plant. Purified heavy chain was digested with a proteinase and resulting peptides were analyzed by LC-ESI-MS. Peaks in the mass spectrum representing glycopeptides were annotated for the type of glycan attached. In this method two glycopeptides are produced as a result of partial inhibition of the proteinase by glycosylation. Therefore, gp-1 and gp-2 refer to a similar heavy chain glycopeptide differing by 1 amino acid. ■ = N-acetylglucosamine (Gn), ● = Mannose (Man) and ◀ = fucose (F). From the glycan analysis it is apparent that xylose residues are not present on the heterologous glycan structures.

Detailed description of the invention

Nicotiana benthamiana has been described as an amphidiploid species from a hybridization between *Nicotiana debneyi* and *Nicotiana suaveolens* (Goodspeed, T. H. (1954) *The Genus Nicotiana*, Waltham, Massachusetts: Chronica Botanica). An amphidiploid is a polyploid formed from the union of two separate chromosome sets and their subsequent doubling, thus *N. benthamiana* can also be designated as an allotetraploid species.

The invention provides in a first embodiment a beta-1,2-xylosyltransferase null mutant of the plant *Nicotiana benthamiana*, or cells, parts, seed or progeny thereof, reference seed having been deposited on May 21, 2009 at the NCIMB under accession number NCIMB 41622. In another embodiment the invention provides a beta-1,2-xylosyltransferase null mutant of the plant *Nicotiana benthamiana*, or cells, parts, seed or progeny thereof, obtained from reference seed having been deposited on May 21, 2009 at the NCIMB under accession number NCIMB 41622. In yet another embodiment the invention provides a beta-1,2-xylosyltransferase null mutant of the plant *Nicotiana benthamiana*, or cells, parts, seed or progeny thereof, obtainable by propagation of and/or breeding with a plant grown from the reference seed having been deposited on May 21, 2009 at the NCIMB under accession number NCIMB 41622. It is envisaged that two "alleles" are present *in vivo* for each beta-1,2-xylosyltransferase gene at each XylT locus in the genome (one allele being the gene sequence found on one chromosome and the other on the homologous chromosome). The nucleotide sequence of these two alleles may be identical (homozygous plant) or different (heterozygous plant) in any given plant, although the number of different possible alleles existing for each *XylT* gene may be much larger than two in the species population as a whole.

In another embodiment a *Nicotiana benthamiana* beta-1,2-xylosyltransferase null mutant plant or plant cell is provided characterized by having a combination of homozygous null alleles selected from a null allele from the group consisting of xyltg14-1, xyltg14-2 or xyltg14-3 and a null allele selected from the group consisting of xyltg19-1 or xyltg19-2.

Reference seeds of *Nicotiana benthamiana* plants comprising alleles xyltg14-1 and xyltg19-1 in homozygous state have been deposited at the National Collection of Industrial, Marine and Food Bacteria (NCIMB), NCIMB Ltd, Ferguson Building, Craibstone Estate, Bucksburn, Aberdeen AB219YA, Scotland, on May 21, 2009, under accession number NCIMB 41622 (strain designation 09GNNB000046).

In another embodiment the invention provides *Nicotiana benthamiana* seed characterized as being homozygous for two null alleles, xyltg14-1 and xyltg19-1, of beta-1,2-xylosyltransferase, having been deposited at the NCIMB on May 21, 2009, under accession number NCIMB 41622.

- 5 In a further embodiment the invention provides a *Nicotiana benthamiana* plant, or a cell, part, seed or progeny thereof, obtained from the seed which has been deposited at the NCIMB on May 21, 2009, under accession number NCIMB 41622.

Definitions:

- 10 In this invention "seed" refers to any plant structure which is formed by continued differentiation of the ovule of the plant, following its normal maturation point at flower opening, irrespective of whether it is formed in the presence or absence of fertilization and irrespective of whether or not said seed structure is fertile or infertile.
- 15 The word "expression" as used herein shall be taken in its widest context to refer to the transcription of a particular genetic sequence to produce sense or antisense mRNA or the translation of a sense mRNA molecule to produce a peptide, polypeptide, oligopeptide, protein or enzyme molecule. In the case of expression comprising the production of a sense mRNA transcript, the word
- 20 "expression" may also be construed to indicate the combination of transcription and translation processes, with or without subsequent post-translational events which modify the biological activity, cellular or sub-cellular localization, turnover or steady-state level of the peptide, polypeptide, oligopeptide, protein or enzyme molecule.
- 25 By "inhibiting, interrupting, knocking-out, knocking-down or otherwise reducing the expression" of a stated integer is meant that transcription and/or translation post-translational modification of the integer is inhibited or prevented or knocked-down or knocked-out or interrupted such that the specified integer has a reduced

biological effect on a cell, tissue, organ or organism in which it would otherwise be expressed.

Those skilled in the art will be aware of how whether expression is inhibited, interrupted or reduced, without undue experimentation. For example, the level of
5 expression of a particular gene may be determined by polymerase chain reaction (PCR) following reverse transcription of an mRNA template molecule. Alternatively, the expression level of a genetic sequence may be determined by northern hybridisation analysis or dot-blot hybridisation analysis or in situ hybridisation analysis or similar technique, wherein mRNA is transferred to a
10 membrane support and hybridised to a "probe" molecule which comprises a nucleotide sequence complementary to the nucleotide sequence of the mRNA transcript encoded by the gene-of-interest, labeled with a suitable reporter molecule such as a radioactively-labelled dNTP (eg [alpha-32P] dCTP or [alpha-35S] dCTP) or biotinylated dNTP, amongst others. Expression of the gene-of-
15 interest may then be determined by detecting the appearance of a signal produced by the reporter molecule bound to the hybridised probe molecule.

Alternatively, the rate of transcription of a particular gene may be determined by nuclear run-on and/or nuclear run-off experiments, wherein nuclei are isolated from a particular cell or tissue and the rate of incorporation of rNTPs into specific
20 mRNA molecules is determined. Alternatively, the expression of the gene-of-interest may be determined by RNase protection assay, wherein a labelled RNA probe or "riboprobe" which is complementary to the nucleotide sequence of mRNA encoded by said gene- of-interest is annealed to said mRNA for a time and under conditions sufficient for a double-stranded mRNA molecule to form,
25 after which time the sample is subjected to digestion by RNase to remove single-stranded RNA molecules and in particular, to remove excess unhybridised riboprobe. Such approaches are described in detail by Sambrook, J., Fritsch, E.F. and Maniatis, T. *Molecular Cloning: a laboratory manual*. 2nd ed. N.Y., Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, 1989. 1659 p.
30 ISBN 0-87969-309-6.

Those skilled in the art will also be aware of various immunological and enzymatic methods for detecting the level of expression of a particular gene at the protein level, for example using rocket immunoelectrophoresis, ELISA, radioimmunoassay and western blot immunoelectrophoresis techniques, amongst others.

As used herein, the term “allele(s)” means any of one or more alternative forms of a gene at a particular locus. In a diploid (or amphidiploid) cell of an organism, alleles of a given gene are located at a specific location or locus (loci plural) on a chromosome. One allele is present on each chromosome of the pair of homologous chromosomes.

As used herein, the term “heterozygous” means a genetic condition existing when two different alleles reside at a specific locus, but are positioned individually on corresponding pairs of homologous chromosomes in the cell. Conversely, as used herein, the term “homozygous” means a genetic condition existing when two identical alleles reside at a specific locus, but are positioned individually on corresponding pairs of homologous chromosomes in the cell.

Whenever reference to a “plant” or “plants” according to the invention is made, it is understood that also plant parts (cells, tissues or organs, seeds, severed parts such as roots, leaves, flowers, pollen, etc.), progeny of the plants which retain the distinguishing characteristics of the parents, such as seed obtained by selfing or crossing, are encompassed herein, unless otherwise indicated.

“Wild type” (also written “wildtype” or “wild-type”), as used herein, refers to a typical form of a plant or a gene as it most commonly occurs in nature. A “wild type plant” refers to a plant with the most common phenotype of such plant in the natural population. A “wild type allele” refers to an allele of a gene required to produce the wild-type phenotype. By contrast, a “mutant plant” refers to a plant with a different rare phenotype of such plant in the natural population or produced

by human intervention, e.g. by mutagenesis, and a “mutant allele” refers to an allele of a gene required to produce the mutant phenotype.

As used herein, the term “wild type *beta-1,2-xylosyltransferase*” (e.g. wild type
5 *XylTg14* or *XylTg19*), means a naturally occurring *XylT* allele found within
Nicotiana, in particular *Nicotiana benthamiana* plants, which encodes a functional
XylT protein. A fragment of the *XylTg14* gene is depicted in SEQ ID NO: 11 in
WO2007107296 and a fragment of the *XylTg19* gene is depicted in SEQ ID NO:
13 in WO2007107296. In the present application the nucleotide sequence of a
10 fragment of the *XylTg14* gene is in the present application depicted as SEQ ID
NO: 1 while the nucleotide sequence of a fragment of the *XylTg19* gene is in the
present application depicted as SEQ ID NO: 3. In contrast, the term “mutant
XylT” (e.g. mutant *XylTg14* or *XylTg19*), as used herein, refers to a *XylT* allele,
which does not encode a functional *XylT* protein, i.e. a *XylT* allele encoding a
15 non-functional *XylT* protein, which, as used herein, refers to a *XylT* protein having
no biological activity or encoding no *XylT* protein at all. Such a “mutant *XylT*
allele” (also herein further designated as “null” allele) is a wild-type *XylT* allele,
which comprises one or more mutations in its nucleic acid sequence, whereby
the mutation(s) result in no detectable amount of functional *XylT* protein in the
20 plant or plant cell *in vivo*. In a preferred embodiment said mutations in the nucleic
acid sequence lead to a STOP codon when said nucleic acid sequence is
translated. As used herein, “a *beta-1,2-xylosyltransferase* null mutant” is a
Nicotiana benthamiana plant with two *XylTg14* null alleles and two *XylTg19* null
alleles which combination results in a loss of *beta-1,2* bound xylose-sugars on
25 endogenous and heterologous produced N-glycan structures of glycoproteins.
Mutant alleles of the *XylT* protein-encoding nucleic acid sequences are
designated as “*xylt*” (e.g. *xyltg14-1*, *xyltg14-2*, *xyltg14-3* or *xyltg19-1*, *xyltg19-2*,
respectively) herein.

Allele *xyltg14-1* corresponds with a C → T mutation on position 192 in SEQ ID
30 NO: 1, thereby introducing a STOP codon in SEQ ID NO: 1.

76766-76

Allele *xyltg14-2* corresponds with a G → A mutation on position 212 in SEQ ID NO: 1, thereby introducing a STOP codon in SEQ ID NO: 1.

Allele *xyltg14-3* corresponds with a G → A mutation on position 329 in SEQ ID NO: 1, thereby introducing a STOP codon in SEQ ID NO: 1.

- 5 Allele *xyltg19-1* corresponds with a C → T mutation on position 139 in SEQ ID NO: 3, thereby introducing a STOP codon in SEQ ID NO: 3.

Allele *xyltg19-2* corresponds with a G → A mutation on position 183 in SEQ ID NO: 3, thereby introducing a STOP codon in SEQ ID NO: 3.

- 10 A summary of the identified alleles and possibility of the occurrence of other alleles is depicted in Table 2.

Mutant null alleles can be either “natural mutant” null alleles, which are mutant null alleles found in nature (e.g. produced spontaneously without human application of mutagens) or “induced mutant” null alleles, which are induced by human intervention, e.g. by mutagenesis and are called non-natural mutant null alleles.

- 15 A “significantly reduced amount of functional XylT protein” refers to a reduction in the amount of a functional XylT protein produced by the cell comprising a mutant *xyltg14* or *xyltg19* allele by at least 95% or preferably 100% (i.e. no functional XylT protein is produced by the alleles) as compared to the amount of the functional XylT protein produced by the cell not comprising the mutant *XylT* alleles. This definition
20 encompasses the production of a “non-functional” XylT protein (e.g. truncated XylT protein) having no biological activity *in vivo*, the reduction in the absolute amount of the functional XylT protein (e.g. no functional XylT protein being made due to the mutation in the *XylT* gene).

- “Mutagenesis”, as used herein, refers to the process in which plant cells
25 (e.g., a plurality of *Nicotiana benthamiana* seeds or other parts, such as pollen, etc.) are subjected to a technique which induces mutations in the DNA of the cells, such as contact with a mutagenic agent, such as a chemical substance (such as

ethylmethylsulfonate (EMS), ethylnitrosourea (ENU), etc.) or ionizing radiation (neutrons (such as in fast neutron mutagenesis, etc.), alpha rays, gamma rays (such as that supplied by a Cobalt 60 source), X-rays, UV-radiation, etc.), or a combination of two or more of these. Thus, the desired mutagenesis of one or more *Xy/T* alleles may be accomplished by use of chemical means such as by contact of one or more plant tissues with ethylmethylsulfonate (EMS), ethylnitrosourea, etc., by the use of physical means such as x-ray, etc, or by gamma radiation, such as that supplied by a Cobalt 60 source. While mutations created by irradiation are often large deletions or other gross lesions such as translocations or complex rearrangements, mutations created by chemical mutagens are often more discrete lesions such as point mutations. For example, EMS alkylates guanine bases, which results in base mispairing: an alkylated guanine will pair with a thymine base, resulting primarily in G/C to A/T transitions. Following mutagenesis, *Nicotiana benthamiana* plants are regenerated from the treated cells using known techniques. For instance, the resulting *Nicotiana benthamiana* seeds may be planted in accordance with conventional growing procedures and following self-pollination seed is formed on the plants. Additional seed that is formed as a result of such self-pollination in the present or a subsequent generation may be harvested and screened for the presence of mutant *Xy/T* alleles. Several techniques are known to screen for specific mutant alleles, e.g., DeleteageneTM (Delete-a-gene; Li *et al.*, 2001, Plant J 27: 235-242) uses polymerase chain reaction (PCR) assays to screen for deletion mutants generated by fast neutron mutagenesis, TILLING (targeted induced local lesions in genomes; McCallum *et al.*, 2000, Nat Biotechnol 18:455-457) identifies EMS-induced point mutations, etc. Additional techniques to screen for the presence of specific mutant *Xy/T* alleles are described in the Examples below.

For the purpose of this invention, the "sequence identity" of two related nucleotide or amino acid sequences, expressed as a percentage, refers to the number of positions in the two optimally aligned sequences which have identical residues (x100) divided by the number of positions compared. A gap, i.e., a

position in an alignment where a residue is present in one sequence but not in the other, is regarded as a position with non-identical residues. The "optimal alignment" of two sequences is found by aligning the two sequences over the entire length according to the Needleman and Wunsch global alignment algorithm (Needleman and Wunsch, 1970, *J Mol Biol* 48(3):443-53) in The European Molecular Biology Open Software Suite (EMBOSS, Rice *et al.*, 2000, *Trends in Genetics* 16(6): 276—277; see e.g. <http://www.ebi.ac.uk/emboss/align/index.html>) using default settings (gap opening penalty = 10 (for nucleotides) / 10 (for proteins) and gap extension penalty = 0.5 (for nucleotides) / 0.5 (for proteins)). For nucleotides the default scoring matrix used is EDNAFULL and for proteins the default scoring matrix is EBLOSUM62.

Nucleic acid sequences according to the invention

Provided are both wild type *XylT* nucleic acid sequences encoding functional *XylT* proteins and mutant *xylt* nucleic acid sequences (comprising one or more mutations, preferably mutations which result in no or a significantly reduced biological activity of the encoded *XylT* protein or in no *XylT* protein being produced) of *XylT* genes from *Nicotiana* species, particularly from *Nicotiana benthamiana*.

Mutant nucleic acid sequences of *Xyltg14* and *Xyltg19* have been isolated from *Nicotiana benthamiana* as depicted in the sequence listing. The wild type *Xyltg14* and *Xyltg19* sequences are depicted, while the mutant *xyltg14* and *xyltg19* sequences of these sequences are described herein below in Figure 2 and in the examples, with reference to the wild type *Xyltg14* and *Xyltg19* sequences.

To determine the functionality of a specific *Xylt* allele/protein in plants, particularly in *Nicotiana benthamiana* plants, the functional level of beta-1,2-xylosyltransferase activity can be determined as described further in the examples. Alternatively *Xylt* alleles can be incorporated into a transformation vector and be used to transform a knock-out line of the model plant *Arabidopsis*

thaliana, with deficiency of active beta-1,2-xylosyltransferase (XylT) and core alpha-1,3-fucosyltransferase (FucT) (Strasser *et al.* (2004) *FEBS Lett.* 561: 132-136). Complementation of this knock-out line with the particular XylT allele should indicate whether the XylT allele is functional or whether it is a null allele.

- 5 Functional complementation as indicated by the presence of xylose-residues on plant or heterologous glycoproteins can be monitored as further shown in the examples.

Methods according to the invention

- 10 Mutant *xylt14* and/or *xylt19* alleles may be generated (for example induced by mutagenesis) and/or identified using a range of methods, which are conventional in the art, for example using PCR based methods to amplify part or all of the *ind* genomic or cDNA.
- 15 Following mutagenesis, plants are grown from the treated seeds, or regenerated from the treated cells using known techniques. For instance, mutagenized seeds may be planted in accordance with conventional growing procedures and following self-pollination seed is formed on the plants. Additional seed which is formed as a result of such self-pollination in the present or a subsequent
- 20 generation may be harvested and screened for the presence of mutant *XylT* alleles, using techniques which are conventional in the art, for example polymerase chain reaction (PCR) based techniques (amplification of the *ind* alleles) or hybridization based techniques, e.g. Southern blot analysis, BAC library screening, and the like, and/or direct sequencing of *xylt* alleles. To screen
- 25 for the presence of point mutations (so called Single Nucleotide Polymorphisms or SNPs) in mutant *IND* alleles, SNP detection methods conventional in the art can be used, for example oligo-ligation-based techniques, single base extension-based techniques or techniques based on differences in restriction sites, such as TILLING.

30

As described above, mutagenization (spontaneous as well as induced) of a specific wild-type *Xy/T* allele results in the presence of one or more deleted, inserted, or substituted nucleotides (hereinafter called "mutation region") in the resulting mutant *Xy/T* allele. The mutant *Xy/T* allele can thus be characterized by

5 the location and the configuration of the one or more deleted, inserted, or substituted nucleotides in the wild type *Xy/T* allele. The site in the wild type *Xy/T* allele where the one or more nucleotides have been inserted, deleted, or substituted, respectively, is herein also referred to as the "mutation region or sequence". A "5' or 3' flanking region or sequence" as used herein refers to a

10 DNA region or sequence in the mutant (or the corresponding wild type) *Xy/T* allele of at least 20 bp, preferably at least 50 bp, at least 750 bp, at least 1500 bp, and up to 5000 bp of DNA different from the DNA containing the one or more deleted, inserted, or substituted nucleotides, preferably DNA from the mutant (or the corresponding wild type) *Xy/T* allele which is located either immediately

15 upstream of and contiguous with (5' flanking region or sequence") or immediately downstream of and contiguous with (3' flanking region or sequence") the mutation region in the mutant *Xy/T* allele (or in the corresponding wild type *XYLT* allele). A "joining region" as used herein refers to a DNA region in the mutant (or the corresponding wild type) *Xy/T* allele where the mutation region and the 5' or

20 3' flanking region are linked to each other. A "sequence spanning the joining region between the mutation region and the 5' or 3' flanking region thus comprises a mutation sequence as well as the flanking sequence contiguous therewith.

The tools developed to identify a specific mutant *Xy/T* allele or the plant or plant

25 material comprising a specific mutant *Xy/T* allele, or products which comprise plant material comprising a specific mutant *Xy/T* allele are based on the specific genomic characteristics of the specific mutant *Xy/T* allele as compared to the genomic characteristics of the corresponding wild type *Xy/T* allele, such as, a specific restriction map of the genomic region comprising the mutation region,

30 molecular markers or the sequence of the flanking and/or mutation regions.

Once a specific mutant *Xy/T* allele has been sequenced, primers and probes can be developed which specifically recognize a sequence within the 5' flanking, 3' flanking and/or mutation regions of the mutant *Xy/T* allele in the nucleic acid (DNA or RNA) of a sample by way of a molecular biological technique. For instance a PCR method can be developed to identify the mutant *Xy/T* allele in biological samples (such as samples of plants, plant material or products comprising plant material). Such a PCR is based on at least two specific "primers": one recognizing a sequence within the 5' or 3' flanking region of the mutant *Xy/T* allele and the other recognizing a sequence within the 3' or 5' flanking region of the mutant *Xy/T* allele, respectively; or one recognizing a sequence within the 5' or 3' flanking region of the mutant *Xy/T* allele and the other recognizing a sequence within the mutation region of the mutant *Xy/T* allele; or one recognizing a sequence within the 5' or 3' flanking region of the mutant *Xy/T* allele and the other recognizing a sequence spanning the joining region between the 3' or 5' flanking region and the mutation region of the specific mutant *Xy/T* allele (as described further below), respectively.

The primers preferably have a sequence of between 15 and 35 nucleotides which under optimized PCR conditions "specifically recognize" a sequence within the 5' or 3' flanking region, a sequence within the mutation region, or a sequence spanning the joining region between the 3' or 5' flanking and mutation regions of the specific mutant *Xy/T* allele, so that a specific fragment ("mutant *Xy/T* specific fragment" or discriminating amplicon) is amplified from a nucleic acid sample comprising the specific mutant *Xy/T* allele. This means that only the targeted mutant *Xy/T* allele, and no other sequence in the plant genome, is amplified under optimized PCR conditions.

Detection and/or identification of a "mutant *Xy/T* specific fragment" can occur in various ways, e.g., via size estimation after gel or capillary electrophoresis or via fluorescence-based detection methods. The mutant *Xy/T* specific fragments may also be directly sequenced. Other sequence specific methods for detection of amplified DNA fragments are also known in the art.

Standard PCR protocols are described in the art, such as in "PCR Applications Manual" (Roche Molecular Biochemicals, 2nd Edition, 1999) and other references. The optimal conditions for the PCR, including the sequence of the specific primers, is specified in a "PCR identification protocol" for each specific mutant *Xy/T* allele. It is however understood that a number of parameters in the PCR identification protocol may need to be adjusted to specific laboratory conditions, and may be modified slightly to obtain similar results. For instance, use of a different method for preparation of DNA may require adjustment of, for instance, the amount of primers, polymerase, $MgCl_2$ concentration or annealing conditions used. Similarly, the selection of other primers may dictate other optimal conditions for the PCR identification protocol. These adjustments will however be apparent to a person skilled in the art, and are furthermore detailed in current PCR application manuals such as the one cited above.

15

Yet other possibilities of PCR identification protocols to identify specific mutant *Xy/T* alleles are described in the Examples section.

Alternatively, specific primers can be used to amplify a mutant *Xy/T* specific fragment that can be used as a "specific probe" for identifying a specific mutant *Xy/T* allele in biological samples. Contacting nucleic acid of a biological sample, with the probe, under conditions that allow hybridization of the probe with its corresponding fragment in the nucleic acid, results in the formation of a nucleic acid/probe hybrid. The formation of this hybrid can be detected (e.g. labeling of the nucleic acid or probe), whereby the formation of this hybrid indicates the presence of the specific mutant *Xy/T* allele. Such identification methods based on hybridization with a specific probe (either on a solid phase carrier or in solution) have been described in the art. The specific probe is preferably a sequence that, under optimized conditions, hybridizes specifically to a region within the 5' or 3' flanking region and/or within the mutation region of the specific mutant *Xy/T* allele (hereinafter referred to as "mutant *Xy/T* specific region"). Preferably, the specific

30

probe comprises a sequence of between 10 and 1000 bp, 50 and 600 bp, between 100 to 500 bp, between 150 to 350bp, which is at least 80%, preferably between 80 and 85%, more preferably between 85 and 90%, especially preferably between 90 and 95%, most preferably between 95% and 100% identical (or complementary) to the nucleotide sequence of a specific region. Preferably, the specific probe will comprise a sequence of about 13 to about 100 contiguous nucleotides identical (or complementary) to a specific region of the specific mutant *Xy/T* allele.

In yet another embodiment the invention provides a *Nicotiana benthamiana* plant which is a double homozygous null mutant for beta-1,2-xylosyltransferase further comprising a silenced alpha-1,3-fucosyltransferase activity.

The level of alfa(1,3) fucosyltransferase activity can be conveniently reduced or eliminated by transcriptional or post-transcriptional silencing of the expression of an endogenous alfa(1,3) fucosyltransferase encoding gene. To this end a silencing RNA molecule is introduced in the plant cells targeting the endogenous alfa(1,3) fucosyltransferase encoding gene. As used herein, "silencing RNA" or "silencing RNA molecule" refers to any RNA molecule, which upon introduction into a plant cell, reduces the expression of a target gene. Such silencing RNA may e.g. be so-called "antisense RNA", whereby the RNA molecule comprises a sequence of at least 20 consecutive nucleotides having 95% sequence identity to the complement of the sequence of the target nucleic acid, preferably the coding sequence of the target gene. However, antisense RNA may also be directed to regulatory sequences of target genes, including the promoter sequences and transcription termination and polyadenylation signals. Silencing RNA further includes so-called "sense RNA" whereby the RNA molecule comprises a sequence of at least 20 consecutive nucleotides having 95% sequence identity to the sequence of the target nucleic acid. Other silencing RNA may be "unpolyadenylated RNA" comprising at least 20 consecutive nucleotides having 95% sequence identity to the complement of the sequence of the target nucleic acid, such as described in WO01/12824 or US6423885

Yet another type of silencing RNA is an RNA molecule as described in WO03/076619 comprising at least 20 consecutive nucleotides having 95% sequence identity to the sequence of the target nucleic acid or the complement thereof, and further

5 comprising a largely-double stranded region as described in WO03/076619 (including largely double stranded regions comprising a nuclear localization signal from a viroid of the Potato spindle tuber viroid-type or comprising CUG trinucleotide repeats). Silencing RNA may also be double stranded RNA comprising a sense and antisense strand as herein defined, wherein the sense

10 and antisense strand are capable of base-pairing with each other to form a double stranded RNA region (preferably the said at least 20 consecutive nucleotides of the sense and antisense RNA are complementary to each other). The sense and antisense region may also be present within one RNA molecule such that a hairpin RNA (hpRNA) can be formed when the sense and antisense

15 region form a double stranded RNA region. hpRNA is well-known within the art (see e.g WO99/53050). The hpRNA may be classified as long hpRNA, having long, sense and antisense regions which can be largely complementary, but need not be entirely complementary (typically larger than about 200 bp, ranging between 200-1000 bp). hpRNA can also be rather small ranging

20 in size from about 30 to about 42 bp, but not much longer than 94 bp (see WO04/073390). Silencing RNA may also be artificial micro-RNA molecules as described e.g. in WO2005/052170, WO2005/047505 or US 2005/0144667.

25 In another embodiment, the silencing RNA molecules are provided to the plant cell or plant by producing a transgenic plant cell or plant comprising a chimeric gene capable of producing a silencing RNA molecule, particularly a double stranded RNA ("dsRNA") molecule, wherein the complementary RNA strands of

30 such a dsRNA molecule comprises a part of a nucleotide sequence encoding a XylT or FucT protein.

The enzyme that catalyses the transfer of fucose from GDP-fucose to the core β -linked N-acetyl glucosamine (GlcNAc) of protein-bound N-glycans is α -1,3-fucosyltransferase ("FucT", EC 2.4.1.214).

- Genes encoding α (1,3) fucosyltransferase (FucT) in plants are well known and include the following database entries identifying experimentally demonstrated and putative FucT cDNA and gene sequences, parts thereof or homologous sequences: NM 112815 (*Arabidopsis thaliana*), NM103858 (*Arabidopsis thaliana*), AJ 618932 (*Physcomitrella patens*) At1g49710(*Arabidopsis thaliana*) and At3g19280 (*Arabidopsis thaliana*). DQ789145 (*Lemna minor*), AY557602 (*Medicago truncatula*) Y18529 (*Vigna radiata*) AP004457 (*Oryza sativa*), AJ891040 encoding protein CAI70373 (*Populus alba* x *Populus tremula*) AY082445 encoding protein AAL99371 (*Medicago sativa*) AJ582182 encoding protein CAE46649 (*Triticum aestivum*) AJ582181 encoding protein CAE46648 (*Hordeum vulgare*).
- Based on the available sequences, the skilled person can isolate genes encoding α (1,3) fucosyltransferase or genes encoding β (1,2) xylosyltransferase from plants other than the plants mentioned above. Homologous nucleotide sequence may be identified and isolated by hybridization under stringent conditions using as probes identified nucleotide sequences.
- "Stringent hybridization conditions" as used herein means that hybridization will generally occur if there is at least 95% and preferably at least 97% sequence identity between the probe and the target sequence. Examples of stringent hybridization conditions are overnight incubation in a solution comprising 50% formamide, 5 x SSC (150 mM NaCl, 15 mM trisodium citrate), 50 mM sodium phosphate (pH 7.6), 5x Denhardt's solution, 10% dextran sulfate, and 20 μ g/ml denatured, sheared carrier DNA such as salmon sperm DNA, followed by washing the hybridization support in 0.1 x SSC at approximately 65 °C, preferably twice for about 10 minutes. Other hybridization and wash conditions are well known and are exemplified in Sambrook et al, Molecular Cloning: A

Laboratory Manual, Second Edition, Cold Spring Harbor, NY (1989), particularly chapter 11.

Nucleotide sequences obtained in this way should be verified for encoding a polypeptide having an amino acid sequence which is at least 80% to 95% identical to a known $\alpha(1,3)$ fucosyltransferase from plants.

In yet another embodiment the β -1,2-xylosyltransferase null mutant *Nicotiana benthamiana* plant which comprises a silenced $\alpha(1,3)$ fucosyltransferase additionally comprises a β -1,4-galactosyltransferase activity. Conveniently, such activity may be introduced into plant cells by providing them with a chimeric gene comprising a plant-expressible promoter operably linked to a DNA region encoding a $\beta(1,4)$ galactosyltransferase and optionally a 3' end region involving in transcription termination and polyadenylation functional in plant cells. The term " β -1,4- galactosyltransferase" refers to the glycosyltransferase designated as EC2.4.1.38 that is required for the biosynthesis of the backbone structure from type 2 chain (Gal β 1 $\rightarrow$ 4GlcNAc), which appears widely on N-linked glycans, i.e., which enzyme has galactosylating activity on N-linked glycans. Useful β -1,4-galactosyltransferases are derived from human, mouse, rat as well as orthologs of β -1,4-galactosyltransferase from non-mammalian species such as chicken and zebrafish (see also WO2008125972).

Regions encoding a β -1,4-galactosyltransferase are preferably obtained from mammalian organisms, including humans, but may be obtained from other organisms as well. NM022305 (*Mus musculus*) NM146045 (*Mus musculus*) NM004776 (*Homo sapiens*) NM 001497(*Homo sapiens*) are a few database entries for genes encoding a $\beta(1,4)$ galactosyltransferase. Others database entries for $\beta(1,4)$ galactosyltransferases include AAB05218 (*Gallus gallus*), XP693272 (*Danio rerio*), CAF95423 (*Tetraodon nigroviridis*) or NP001016664 (*Xenopus tropicalis*).

As used herein, the term "plant-expressible promoter" means a DNA sequence that is capable of controlling (initiating) transcription in a plant cell. This includes

any promoter of plant origin, but also any promoter of non-plant origin which is capable of directing transcription in a plant cell, i.e., certain promoters of viral or bacterial origin such as the CaMV35S (Harpster *et al.* (1988) *Mol Gen Genet.* 212(1):182-90, the subterranean clover virus promoter No 4 or No 7
5 (WO9606932), or T-DNA gene promoters but also tissue-specific or organ-specific promoters including but not limited to seed-specific promoters (e.g., WO89/03887), organ-primordia specific promoters (An *et al.* (1996) *Plant Cell* 8(1):15-30), stem-specific promoters (Keller *et al.*, (1988) *EMBO J.* 7(12): 3625-3633), leaf specific promoters (Hudspeth *et al.* (1989) *Plant Mol Biol.* 12: 579-
10 589), mesophyl-specific promoters (such as the light-inducible Rubisco promoters), root-specific promoters (Keller *et al.* (1989) *Genes Dev.* 3: 1639-1646), tuber-specific promoters (Keil *et al.* (1989) *EMBO J.* 8(5): 1323-1330), vascular tissue specific promoters (Peleman *et al.* (1989) *Gene* 84: 359-369), stamen-selective promoters (WO 89/10396, WO 92/13956), dehiscence zone
15 specific promoters (WO 97/13865) and the like.

In yet another embodiment the mutant *Nicotiana benthamiana* plants comprising a silenced fucosyltransferase and optionally a further beta-1,4-galactosyltransferase can comprise also a heterologous gene encoding a glycoprotein. The glycoproteins may be glycoproteins endogeneous to the cell of
20 the higher plant, and may result in altered functionality, folding or half-life of these proteins. Glycoproteins also include proteins which are foreign to the cell of the higher plant (i.e. a heterologous glycoprotein), i.e. which are not normally expressed in such plant cells in nature. These may include mammalian or human proteins, which can be used as therapeutics such as e.g. monoclonal antibodies.
25 Conveniently, the foreign glycoproteins may be expressed from chimeric genes comprising a plant-expressible promoter and the coding region of the glycoprotein of interest, whereby the chimeric gene is stably integrated in the genome of the plant cell. Methods to express foreign proteins in plant cells are well known in the art. Alternatively, the foreign glycoproteins may also be
30 expressed in a transient manner, e.g. using the viral vectors and methods described in WO02/088369, WO2006/079546 and WO2006/012906 or using the

viral vectors described in WO89/08145, WO93/03161 and WO96/40867 or WO96/12028.

By "heterologous protein" it is understood a protein (i.e. a polypeptide) that is not expressed by the plant or plant cells in nature. This is in contrast with a
5 homologous protein which is a protein naturally expressed by a plant or plant cell. Heterologous and homologous polypeptides that undergo post-translational N-glycosylation are referred to herein as heterologous or homologous glycoproteins.

Examples of heterologous proteins of interest that can be advantageously produced by the methods of this invention include, without limitation, cytokines,
10 cytokine receptors, growth factors (e.g. EGF, HER-2, FGF-alpha, FGF-beta, TGF-alpha, TGF-beta, PDGF, IGF-I, IGF-2, NGF), growth factor receptors. Other examples include growth hormones (e.g. human growth hormone, bovine growth hormone); insulin (e.g., insulin A chain and insulin B chain), pro-insulin, erythropoietin (EPO), colony stimulating factors (e.g. G-CSF, GM-CSF, M-CSF);
15 interleukins; vascular endothelial growth factor (VEGF) and its receptor (VEGF-R), interferons, tumor necrosis factor and its receptors, thrombopoietin (TPO), thrombin, brain natriuretic peptide (BNP); clotting factors (e.g. Factor VIII, Factor IX, von Willebrands factor and the like), anti-clotting factors; tissue plasminogen activator (TPA), urokinase, follicle stimulating hormone (FSH), luteinizing
20 hormone (LH), calcitonin, CD proteins (e. g., CD2, CD3, CD4, CD5, CD7, CD8, CDI Ia, CDI Ib, CD18, CD19, CD20, CD25, CD33, CD44, CD45, CD71, etc.), CTLA proteins (e.g. CTLA4); T-cell and B-cell receptor proteins, bone morphogenic proteins (BNPs, e.g. BMP-I, BMP-2, BMP-3, etc.), neurotrophic factors, e.g. bone derived neurotrophic factor (BDNF), neurotrophins, e.g. rennin,
25 rheumatoid factor, RANTES, albumin, relaxin, macrophage inhibitory protein (e.g. MIP-I, MIP-2), viral proteins or antigens, surface membrane proteins, ion channel proteins, enzymes, regulatory proteins, immunomodulatory proteins, (e.g. HLA, MHC, the B7 family), homing receptors, transport proteins, superoxide dismutase (SOD), G-protein coupled receptor proteins (GPCRs), neuromodulatory proteins,
30 Alzheimer's Disease associated proteins and peptides. Fusion proteins and

polypeptides, chimeric proteins and polypeptides, as well as fragments or portions, or mutants, variants, or analogs of any of the aforementioned proteins and polypeptides are also included among the suitable proteins, polypeptides and peptides that can be produced by the methods of the present invention. In a preferred embodiment, the protein of interest is a glycoprotein. One class of glycoproteins are viral glycoproteins, in particular subunits, than can be used to produce for example a vaccine. Some examples of viral proteins comprise proteins from rhinovirus, poliomyelitis virus, herpes virus, bovine herpes virus, influenza virus, newcastle disease virus, respiratory syncytio virus, measles virus, retrovirus, such as human immunodeficiency virus or a parvovirus or a papovavirus, rotavirus or a coronavirus, such as transmissible gastroenteritisvirus or a flavivirus, such as tick-borne encephalitis virus or yellow fever virus, a togavirus, such as rubella virus or eastern-, western-, or venezuelean equine encephalomyelitis virus, a hepatitis causing virus, such as hepatitis A or hepatitis B virus, a pestivirus, such as hog cholera virus or a rhabdovirus, such as rabies virus. In another preferred embodiment, the heterologous glycoprotein is an antibody or a fragment thereof. The term "antibody" refers to recombinant antibodies (for example of the classes IgD, IgG, IgA, IgM, IgE) and recombinant antibodies such as single-chain antibodies, chimeric and humanized antibodies and multi-specific antibodies. The term "antibody" also refers to fragments and derivatives of all of the foregoing, and may further comprises any modified or derivatised variants thereof that retain the ability to specifically bind an epitope. Antibody derivatives may comprise a protein or chemical moiety conjugated to an antibody. A monoclonal antibody is capable of selectively binding to a target antigen or epitope. Antibodies include, monoclonal antibodies (mAbs), humanized or chimeric antibodies, camelized antibodies, camelid antibodies (nanobodies[®]), single chain antibodies (scFvs), Fab fragments, F(ab')₂ fragments, disulfide- linked Fvs (sdFv) fragments, anti-idiotypic (anti-Id) antibodies, intra-bodies, synthetic antibodies, and epitope-binding fragments of any of the above. The term "antibody" also refers to fusion protein that includes a region equivalent to the Fc region of an immunoglobulin.

Also envisaged is the production in the plant or plant cells of the invention of so called dual-specificity antibodies (Bostrom J *et al* (2009) *Science* 323, 1610-1614).

Preferred antibodies within the scope of the present invention include those
5 comprising the amino acid sequences of the following antibodies: anti-HER2
antibodies including antibodies comprising the heavy and light chain variable
regions (see US5,725,856) or Trastuzumab such as HERCEPTIN™; anti-CD20
antibodies such as chimeric anti-CD20 as in US5,736,137, a chimeric or
humanized variant of the 2H7 antibody as in US5,721,108; anti-VEGF antibodies
10 including humanized and/or affinity matured anti-VEGF antibodies such as the
humanized anti- VEGF antibody huA4.6.1 AVASTIN™ (WO 96/30046 and WO
98/45331); anti-EGFR (chimerized or humanized antibody as in WO 96/40210);
anti-CD3 antibodies such as OKT3 (US4,515,893); anti-CD25 or anti-tac
antibodies such as CHI-621 (SIMULECT) and (ZENAPAX) (US5,693,762). The
15 present invention provides a method for the production of an antibody which
comprises culturing a transformed plant cell or growing a transformed plant of the
present invention. The produced antibody may be purified and formulated in
accordance with standard procedures.

The nucleotide sequences of the glycosyltransferases and/or the heterologous
20 genes may be codon optimized to increase the level of expression within the
plant. By codon optimization it is meant the selection of appropriate DNA
nucleotides for the synthesis of oligonucleotide building blocks, and their
subsequent enzymatic assembly, of a structural gene or fragment thereof in order
to approach codon usage in plants.

25 In certain embodiments methods for obtaining a desired glycoprotein or
functional fragment thereof comprise cultivating a plant described herein until
said plant has reached a harvestable stage, harvesting and fractionating the plant
to obtain fractionated plant material and at least partly isolating said glycoprotein
from said fractionated plant material.

In certain embodiments methods for obtaining a desired glycoprotein or functional fragment thereof comprise growing recombinant plant cells in cell culture in a fermentor until said cell culture has reached a harvestable stage or the desired glycoprotein can be collected from the medium. The glycoproteins described herein, such as e.g., antibodies, vaccines, cytokines and hormones, may be purified by standard techniques well known to those of skill in the art. Such recombinantly produced proteins may be directly expressed or expressed as a fusion protein. The recombinant protein is purified by a combination of cell lysis (e.g., sonication, French press) and affinity chromatography or other affinity-based method. For fusion products, subsequent digestion of the fusion protein with an appropriate proteolytic enzyme releases the desired recombinant protein.

In yet another embodiment the invention provides a method to produce at least one heterologous protein in null mutant *Nicotiana benthamiana* plants described herein before comprising the steps of: a) providing a null mutant plant or plant cell according to the invention with at least one chimeric gene comprising the following operably linked nucleic acid molecules: i) plant-expressible promoter, ii) DNA region encoding a heterologous protein, and iii) DNA region involved in transcription termination and polyadenylation , and b) cultivating said plant or plant cell and isolating said at least one heterologous protein from said plant or plant cell. In a preferred embodiment the heterologous protein produced is an antibody.

The proteins described herein, recombinant or synthetic, may be purified to substantial purity by standard techniques well known in the art, including detergent solubilization, selective precipitation with such substances as ammonium sulfate, column chromatography, immunopurification methods, and others. See, for instance, R. Scopes, Protein Purification: Principles and Practice, Springer- Verlag: New York (1982); Deutscher, Guide to Protein Purification, Academic Press (1990). For example, antibodies may be raised to the proteins as described herein. Purification from *E. coli* can be achieved following procedures described in U. S. Patent No. 4,511,503. The protein may then be

isolated from cells expressing the protein and further purified by standard protein chemistry techniques as described herein. Detection of the expressed protein is achieved by methods known in the art and include, for example, radioimmunoassays, Western blotting techniques or immunoprecipitation.

- 5 Throughout the description and Examples, reference is made to the following sequences:

SEQ ID NO 1: nucleotide sequence of beta-1,2-xylosyltransferase XylTg14 of *Nicotiana benthamiana*

10 SEQ ID NO 2: amino acid sequence of SEQ ID NO: 1

SEQ ID NO 3: nucleotide sequence of beta-1,2-xylosyltransferase XylTg19 of *Nicotiana benthamiana*

SEQ ID NO 4: amino acid sequence of SEQ ID NO: 3

15

Examples

1. Determination of the optimal EMS dosage for M2 seed production

- 20 The optimum dose for EMS mutagenesis was determined by treating seeds of *N. benthamiana* with 0, 50, 75, 100, 150, and 200 mM EMS. Briefly, seeds were imbibed for 2 hours at room temperature, treated with EMS for 4 hours at room temperature and washed 5 times for 15 minutes at room temperature. Seeds were dried overnight and sown immediately. The effects on germination, seedling
- 25 lethality and plant fertility were recorded. *N. benthamiana* has been described to be an amphidiploid species from a combination of *N. debneyi* and *N. suaveolens* (Goodspeed, T. H. (1954) The genus *Nicotiana*, Waltham, Massachusetts: *Chronica Botanica*). Surprisingly, we discovered that the parents, being diploids, proved to be more resistant to EMS as compared to *N. benthamiana*, being a
- 30 tetraploid. Results for the *N. benthamiana* seeds are shown in Figures 1A and 1B. Although EMS treatment caused a delay in germination, no lethality was detected

up to 75 mM EMS. At higher EMS doses, lethality rose quickly and at 150 mM EMS no seeds survived the treatment (Figure 1A). Fertility already was affected at 50 mM. By treating the seeds with 75 mM approximately 60 % of the M1 plants were infertile (Figure 1B). Based on these results, the optimum EMS dose was
5 set at 75 mM.

2. SNP detection by direct sequencing

EMS-induced point mutations were detected in a high-throughput manner by direct sequence analysis by using the method described in Smits B.M. *et al* (2006)
10 *Pharmacogenet. Genomics* 16: 159-169. Briefly, specific gene fragments were amplified by PCR from DNA of leaf tissue of individual plants using gene specific primers. Each primer carried an additional sequence at its 5' end that allowed analysis of the sequence of both strands of the resulting PCR fragment. In a first step we optimized the method. Thereto DNA was extracted from several *N.*
15 *benthamiana* accessions (see Table 1 for the accessions). The first exons of genes *XylTg14* and *XylTg19* were amplified and nucleotide sequences were determined. The chromatograms of sequences were analyzed for Single Nucleotide Polymorphisms (SNPs) by comparing them to the *XylTg14* and *XylTg19* sequences in NovoSNP (Weckx S. *et al.* (2005) *Genome Research*
20 15:436-442). It appeared that only accession NBNPGS2 from the USDA National Germplasm System (accession code PI555684) contained several SNPs compared to the *N. benthamiana* accession used in our research (i.e. Cultivar "BENTHAMIANA" supplied by Icon Genetics GmbH). The position of the identified SNPs are summarized in Figure 2.

25 Accession NBNPGS2 was used as a control and position marker (i.e. leaf material from NBNPGS2 was sampled in well H12) when sampling leaf material, in 96-well format, for DNA production of the final M2 populations.

CULTIVAR NAME	SUPPLIER	SUPPLIER CODE	seed lot number	protocol name
BENTHAMIANA	Icon Genetics		05GANB000001	
NBNIJM1	University of Nijmegen	A34750397	06GNNB000004	06GNNB002
NBNIJM2	University of Nijmegen	964750110	06GNNB000005	06GNNB002
NBNIJM2	University of Nijmegen	964750110	06GNNB000007	06GNNB003
NBNIJM2	University of Nijmegen	964750110	06GNNB000008	06GNNB003
NBNIJM2	University of Nijmegen	964750110	06GNNB000009	06GNNB003
NBVIENNA1	University of Vienna		06GNNB000006	06GNNB003
NBNPGS1	USDA, National Plant Germplasm System	PI555478	06GNNB000010	06GNNB006
NBNPGS2	USDA, National Plant Germplasm System	PI555684	06GNNB000011	06GNNB006
NBALTADIS	Altadis, Institut du Tabac	N. BENTHAMIANA G	06GNNB000012	06GNNB011
NBTRCF1	AusPGRIS - Australian Plant Genetic Resource Information Service	AusTRCF303915	06GNNB000013	06GNNB011
NBTRCF2	AusPGRIS - Australian Plant Genetic Resource Information Service	AusTRCF303916	06GNNB000014	06GNNB011

Table 1. *N. benthamiana* accessions used in the testing and production of EMS-mutagenized M2 population. Accession BENTHAMIANA was used for the final M2 populations. The other accessions were merely used for testing and optimizing the SNP detection method. Accession NBNPGS2 was used as a control in each 96-well plate in sampling the final M2 populations.

3. Defining the target area for mutagenesis detection

- Because the SNP detection by direct sequencing, was limited to sequence fragments of 500 bp, it was necessary to identify a 500 bp region in the XylTg14 and XylTg19 genes that had the highest chance producing a null mutation when mutagenized with EMS. Therefore we needed to identify a region that: (1) had the highest density of codons that can change into stop codons by one G to A or C to T mutation and/or splice donor and acceptor sites, and (2) was placed in or upstream of a catalytic or conserved domain. In order to find the highest density of candidate stop or splice mutations, we used EmsPred; a proprietary algorithm to Bayer BioScience that identifies all codons in a coding sequence that can be

mutated to a stop codon or a splice mutant induced by EMS mutagenesis. The positions identified in XylTg14 and XylTg19 are listed in Table 2. For the identification of a region upstream of a catalytic domain, we used a publication by Pagny et al. (Pagny, S. *et al* (2003) *Plant J.* 33:189-203) which describes the inactivation of *A. thaliana* β -1,2-xylosyltransferase by the removal of 82, 106, and 143 amino acids at the N-terminus. In conclusion, we decided to search for putative null mutations between positions 120 and 720 of both XylTg14 and XylTg19. In XylTg14, this area corresponds to a part of the first exon (until position 650) and part of the first intron and codes for amino acids 41 to 217. In XylTg19, this area also corresponds to part of the first exon (until position 648) and part of the first intron and codes for amino acids 41 to 216.

XylTg14				XylTg19			
position	base	type	allele	position	base	type	allele
84	C	stop		79	C	stop	
144	C	stop		139	C	stop	XylTg19-1
160	G	stop		155	G	stop	
161	G	stop		156	G	stop	
187	G	stop		182	G	stop	
188	G	stop		183	G	stop	XylTg19-2
192	C	stop	XylTg14-1	187	C	stop	
211	G	stop		206	G	stop	
212	G	stop	XylTg14-2	207	G	stop	
328	G	stop		323	G	stop	
329	G	stop	XylTg14-3	324	G	stop	
357	C	stop		352	C	stop	
387	C	stop		382	C	stop	
495	C	stop		490	C	stop	
618	C	stop		613	C	stop	
653	G	splice		652	G	splice	
654	G	splice		653	G	splice	
1913	G	splice		2198	G	splice	
1915	G	stop		2196	G	stop	
1916	G	stop		2197	G	stop	
1944	C	stop		2225	C	stop	
1984	G	stop		2265	G	stop	
1985	G	stop		2266	G	stop	
2063	G	splice		2344	G	splice	
2064	G	splice		2345	G	splice	
2608	G	splice		2888	G	splice	
2612	C	stop		2892	C	stop	
2628	G	stop		2908	G	stop	

2629	G	stop		2909	G	stop	
2784	G	stop		3064	G	stop	
2785	G	stop		3065	G	stop	
2786	C	stop		3066	C	stop	
2870	C	stop		3150	C	stop	
2960	C	stop		3240	C	stop	
2984	C	stop		3289	G	stop	
3009	G	stop		3290	G	stop	
3010	G	stop		3360	C	stop	
3080	C	stop		3366	C	stop	
3086	C	stop		3375	C	stop	
3095	C	stop		3504	C	stop	
3224	C	stop		3508	G	stop	
3228	G	stop		3509	G	stop	
3229	G	stop					

Table 2. Summary of all positions in XylTg14 and -19 that produce stop or splice mutations by one EMS mutation. The positions of the stop codons of the final XylTg14 and -19 alleles are also indicated.

5

4. Identification of XylTg14 and XylTg19 single knock plants and generation of double knock put plants

In total, 5700 M2 individuals were screened for mutations in XylTg14 and 6200 for XylTg19. Three putative null alleles were identified in XylTg14, at nucleotide positions 192, 212, and 392, labeled XylTg14-1, -2, and -3, respectively. Two putative null alleles were identified in XylTg19, at nucleotide positions 139 and 183, labeled XylTg19-1 and -2, respectively (Figure 2 and Table 2).

In order to retrieve homozygous mutants for these mutations, 24 plants from the original M2 seed lot --in which the mutation had been identified-- were grown, sampled, and analyzed for the specific mutation by direct sequencing. Mutants xyltg14-1 were crossed with xyltg19-1 to produce heterozygous double mutants. In addition, all mutants (i.e. including xyltg14-2 and xyltg14-3 and xyltg19-2) were allowed to self fertilize to establish homozygous single mutant seed lots.

Progeny from the XylTg14-1 x XylTg19-1 crosses were analyzed by direct sequencing to confirm their heterozygous genotype and selected plants were allowed to self-fertilize. Double homozygous mutants were identified from the

progeny of these plants by direct sequencing. To establish a stable homozygous seed lot, these plants were allowed to self-fertilize. Simultaneously, these plants were backcrossed with the BENTHAMIANA accession to produce start producing a plant homozygous for xyltg14-1 and xyltg19-1 but free of undesired background mutations.

5. Glycan analysis of XylTg14 and XylTg19 single and double mutants

To determine whether the mutations found in alleles XylTg14-1 and -19-1 cause inactivation of the XylTg14 and -19 genes respectively, a western blot was performed on total protein from different heterozygous and homozygous single and double mutants. 10 µg total protein was loaded per lane, blotted and probed with either anti-xylose or anti-fucose antibodies as produced by the method described by Faye et al. (Faye, L. *et al* (1993) *Anal. Biochem.* 209:104-108). Figure 3 shows that total protein of the double homozygous mutant is not recognized by the anti-xylose antibody. In contrast, protein from either single homozygous mutants or double heterozygous mutants is recognized by the anti-xylose antibody. The control blot probed with the anti-fucose antibody shows that protein was loaded in all lanes. Together this shows that the mutations in alleles xyltg14-1 and xyltg19-1 are null mutations and that generating null mutants of both XylTg14 and XylTg19, for instance in the double homozygous xyltg14-1 and xyltg19-1 plants, is both sufficient and necessary to inactivate the complete β 1,2-xylosyltransferase activity and to fully prevent addition of any β 1,2-xylose to the N-glycans of *N. benthamiana*.

In a next step we investigated the presence or absence of xylose sugars on N-glycans of a heterologous glycoprotein produced in the xyltg14-1 and xyltg19-1 homozygous *N. benthamiana* plant. Thereto, we analyzed the N-glycans present on the heavy chain of an IgG1 expressed in a double knock out plant using magnICON® (Marillonnet *et al.* (2005) *Nature Biotechnology* 23, 718-723). Nine days after infiltration, total protein was extracted from the mutant plant and IgG1 was purified using protein G. The heavy chain of the purified antibody was isolated by cutting the corresponding band from a reducing SDS-PAGE. The

heavy chain protein in this band was used for glycan analysis by LC-MS as described by Kolarich et al. (*Kolarich, D. et al (2006) Proteomics 6:3369-3380*). Results shown in Figure 4 show that no xylose is present on the heavy chain of this IgG1. This confirms that the double homozygous xyltg14-1 and xyltg19-1 *N. benthamiana* mutant completely lacks beta-1,2-xylosyltransferase activity.

5

SEQUENCE LISTING IN ELECTRONIC FORM

In accordance with Section 111(1) of the Patent Rules, this description contains a sequence listing in electronic form in ASCII text format (file: 75749-68 Seq 29-11-11 v1.txt).

A copy of the sequence listing in electronic form is available from the Canadian Intellectual Property Office.

The sequences in the sequence listing in electronic form are reproduced in the following table.

SEQUENCE TABLE

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Ser Ser His Pro Asp His Ser Arg Arg Lys Ser Pro Gln Asn His Phe
              20              25              30

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Ser Ser Ser Glu Asn His His His Asn Phe His Ser Ser Ile Thr Ser
              35              40              45

caa tat tcc agg cct tgg cct att ttg ccc tcc tac ctc cct tgg tct      191
Gln Tyr Ser Arg Pro Trp Pro Ile Leu Pro Ser Tyr Leu Pro Trp Ser
              50              55              60

caa aac cct aat gtt gct tgg aga tca tgc gag ggt tac ttc ggt aat      239
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    80              85              90              95

cgg aaa ttc ggc gaa aac acc gtc ttc gga gac ggc gga tgg ttt agg      335
Arg Lys Phe Gly Glu Asn Thr Val Phe Gly Asp Gly Gly Trp Phe Arg
              100              105              110

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Cys Phe Phe Ser Glu Thr Leu Gln Ser Ser Ile Cys Glu Gly Gly Ala
              115              120              125

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              130              135              140

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Leu Glu Ser Val Ile Gly Arg Ser Glu Asp Asp Glu Val Pro Ala Phe
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 Phe Ser Ser His Pro Asp His Lys Ser Pro Gln Asn His Phe Ser Leu
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 Ser Glu Asn His His His Asn Phe His Ser Ser Ile Thr Ser Gln Tyr
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 Ser Lys Pro Trp Pro Ile Leu Pro Ser Tyr Leu Pro Trp Ser Gln Asn
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Phe Gly Asp Asn Thr Val Ser Gly Asp Gly Gly Trp Phe Arg Cys Phe	
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Ala	Tyr	Ala	Ala	Ser	Arg	Val	Thr	Gly	Leu	Pro	Ser	Arg	Pro	Asn	Leu	
				245					250					255		
Val	Phe	Val	Asp	Gly	His	Cys	Glu	Thr	Gln	Leu	Glu	Glu	Thr	Trp	Lys	
			260					265					270			
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				485					490							

CLAIMS:

1. A cell of a beta-1,2-xylosyltransferase null mutant of the plant *Nicotiana benthamiana*, or progeny cell thereof, said null mutant being obtained by breeding with a plant grown from reference seed having been deposited on May 21, 2009 at the NCIMB under accession number NCIMB 41622, wherein said cell comprises null alleles xyltg14-1 and xyltg19-1 in homozygous state; wherein allele xyltg14-1 comprises a C → T mutation corresponding to position 192 in SEQ ID NO: 1, thereby introducing a STOP codon; and allele xyltg19-1 comprises a C → T mutation corresponding to position 139 in SEQ ID NO: 3, thereby introducing a STOP codon.
2. A *Nicotiana benthamiana* beta-1,2-xylosyltransferase null mutant plant cell comprising a combination of homozygous null alleles:
 - a null allele selected from xyltg14-1, xyltg14-2 or xyltg14-3; and
 - a null allele selected from xyltg19-1 or xyltg19-2;
 - wherein allele xyltg14-1 comprises a C → T mutation corresponding to position 192 in SEQ ID NO: 1, thereby introducing a STOP codon;
 - wherein allele xyltg14-2 comprises a G → A mutation corresponding to position 212 in SEQ ID NO: 1, thereby introducing a STOP codon;
 - wherein allele xyltg14-3 comprises a G → A mutation corresponding to position 329 in SEQ ID NO: 1, thereby introducing a STOP codon;
 - wherein allele xyltg19-1 comprises a C → T mutation corresponding to position 139 in SEQ ID NO: 3, thereby introducing a STOP codon; and
 - wherein allele xyltg19-2 comprises a G → A mutation corresponding to position 183 in SEQ ID NO: 3, thereby introducing a STOP codon.

3. A *Nicotiana benthamiana* plant cell, comprising a combination of homozygous null alleles:

a null allele selected from xyltg14-1, xyltg14-2 or xyltg14-3; and

a null allele selected from xyltg19-1 or xyltg19-2;

5 wherein allele xyltg14-1 comprises a C → T mutation corresponding to position 192 in SEQ ID NO: 1, thereby introducing a STOP codon;

wherein allele xyltg14-2 comprises a G → A mutation corresponding to position 212 in SEQ ID NO: 1, thereby introducing a STOP codon;

10 wherein allele xyltg14-3 comprises a G → A mutation corresponding to position 329 in SEQ ID NO: 1, thereby introducing a STOP codon;

wherein allele xyltg19-1 comprises a C → T mutation corresponding to position 139 in SEQ ID NO: 3, thereby introducing a STOP codon; and

wherein allele xyltg19-2 comprises a G → A mutation corresponding to position 183 in SEQ ID NO: 3, thereby introducing a STOP codon.

15 4. The plant cell according to any one of claims 1 to 3, which does not form beta-1,2-xylosyl-sugars on N-glycan structures of glycoproteins produced in said plant cell.

5. A cell of a *Nicotiana benthamiana* seed, wherein the cell is homozygous for two null alleles, xyltg14-1 and xyltg19-1, of beta-1,2-xylosyltransferase, the seed
20 having been deposited at the NCIMB on May 21, 2009, under accession number NCIMB 41622, wherein allele xyltg14-1 comprises a C → T mutation corresponding to position 192 in SEQ ID NO: 1, thereby introducing a STOP codon; and allele xyltg19-1 comprises a C → T mutation corresponding to position 139 in SEQ ID NO: 3, thereby introducing a STOP codon.

6. A *Nicotiana benthamiana* plant cell, or progeny cell obtained from seed deposited at the NCIMB on May 21, 2009, under accession number NCIMB 41622, wherein the plant cell or progeny cell is homozygous for two null alleles, xyltg14-1 and xyltg19-1 of beta-1,2-xylosyltransferase, wherein allele xyltg14-1 comprises a
 5 C → T mutation corresponding to position 192 in SEQ ID NO: 1, thereby introducing a STOP codon; and allele xyltg19-1 comprises a C → T mutation corresponding to position 139 in SEQ ID NO: 3, thereby introducing a STOP codon.

7. The cell according to any one of claims 1 to 6, further comprising silenced alpha-1,3-fucosyltransferase activity.

10 8. The cell according to claim 7 further comprising a chimeric gene encoding beta-1,4-galactosyltransferase activity.

9. The cell according to any one of claims 1 to 8, further comprising a chimeric gene encoding a heterologous protein.

10. Use of the cell according to any one of claims 1 to 9, or of a plant
 15 comprising the plant cell according to any one of claims 1 to 4 and 6 to 9, or of a seed comprising the seed cell according to claim 5, for the production of a heterologous protein.

11. A method to produce at least one heterologous protein in plants or plant cells, comprising the steps of:

20 a. providing a plant or plant cell with at least one chimeric gene comprising the following operably linked nucleic acid molecules:

(a) a plant-expressible promoter,

(b) a DNA region encoding a heterologous protein, and

(c) a DNA region involved in transcription termination and
 25 polyadenylation, and

b. cultivating said plant or plant cell and isolating said at least one heterologous protein from said plant or plant cell;

wherein said plant or plant cell of item a. is

- (i) a *Nicotiana benthamiana* beta-1,2-xylosyltransferase null mutant
5 plant or cell or progeny thereof, wherein the null mutant plant or cell is obtained by breeding with a plant grown from reference seed having been deposited on May 21, 2009 at the NCIMB under accession number NCIMB 41622, wherein said null mutant plant or cell or progeny thereof comprises null alleles xyltg14-1 and xyltg19-1 in homozygous state;
- 10 (ii) a *Nicotiana benthamiana* beta-1,2-xylosyltransferase null mutant plant or plant cell, comprising a combination of homozygous null alleles: a null allele selected from xyltg14-1, xyltg14-2 or xyltg14-3, and a null allele selected from xyltg19-1 or xyltg19-2;
- (iii) a *Nicotiana benthamiana* plant or cell, comprising a combination of
15 homozygous null alleles: a null allele selected from xyltg14-1, xyltg14-2 or xyltg14-3, and a null allele selected from xyltg19-1 or xyltg19-2; or
- (iv) a *Nicotiana benthamiana* plant or cell or progeny thereof, wherein the plant or cell is obtained from the seed having been deposited at the NCIMB on
20 May 21, 2009, under accession number NCIMB 41622, said plant or cell or progeny thereof being homozygous for two null alleles, xyltg14-1 and xyltg19-1, of beta-1,2-xylosyltransferase;
- wherein allele xyltg14-1 comprises a C → T mutation corresponding to position 192 in SEQ ID NO: 1, thereby introducing a STOP codon;
- wherein allele xyltg14-2 comprises a G → A mutation corresponding to
25 position 212 in SEQ ID NO: 1, thereby introducing a STOP codon;

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wherein allele xyltg14-3 comprises a G → A mutation corresponding to position 329 in SEQ ID NO: 1, thereby introducing a STOP codon;

wherein allele xyltg19-1 comprises a C → T mutation corresponding to position 139 in SEQ ID NO: 3, thereby introducing a STOP codon;

- 5 wherein allele xyltg19-2 comprises a G → A mutation corresponding to position 183 in SEQ ID NO: 3, thereby introducing a STOP codon.

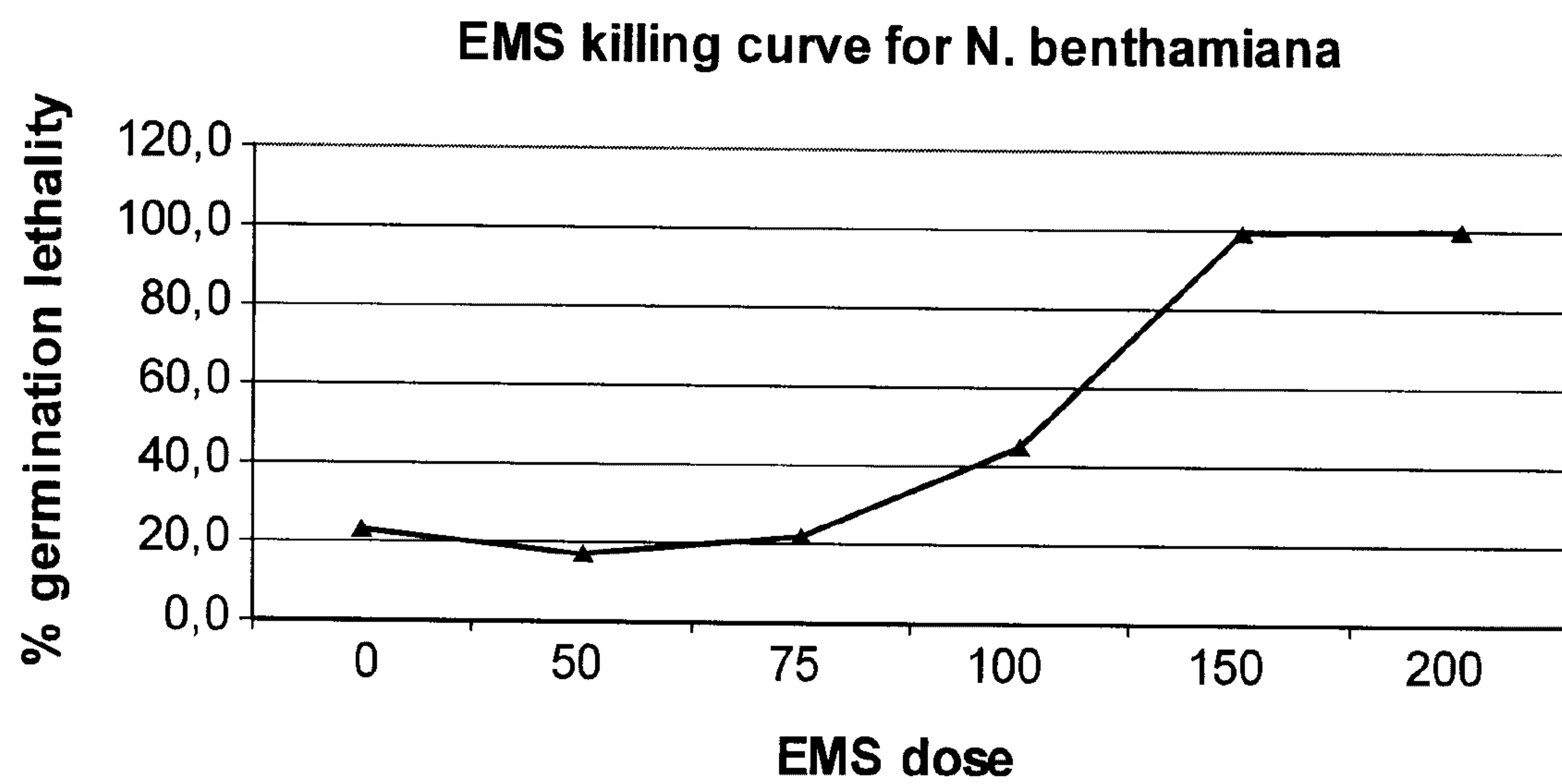


Figure 1A

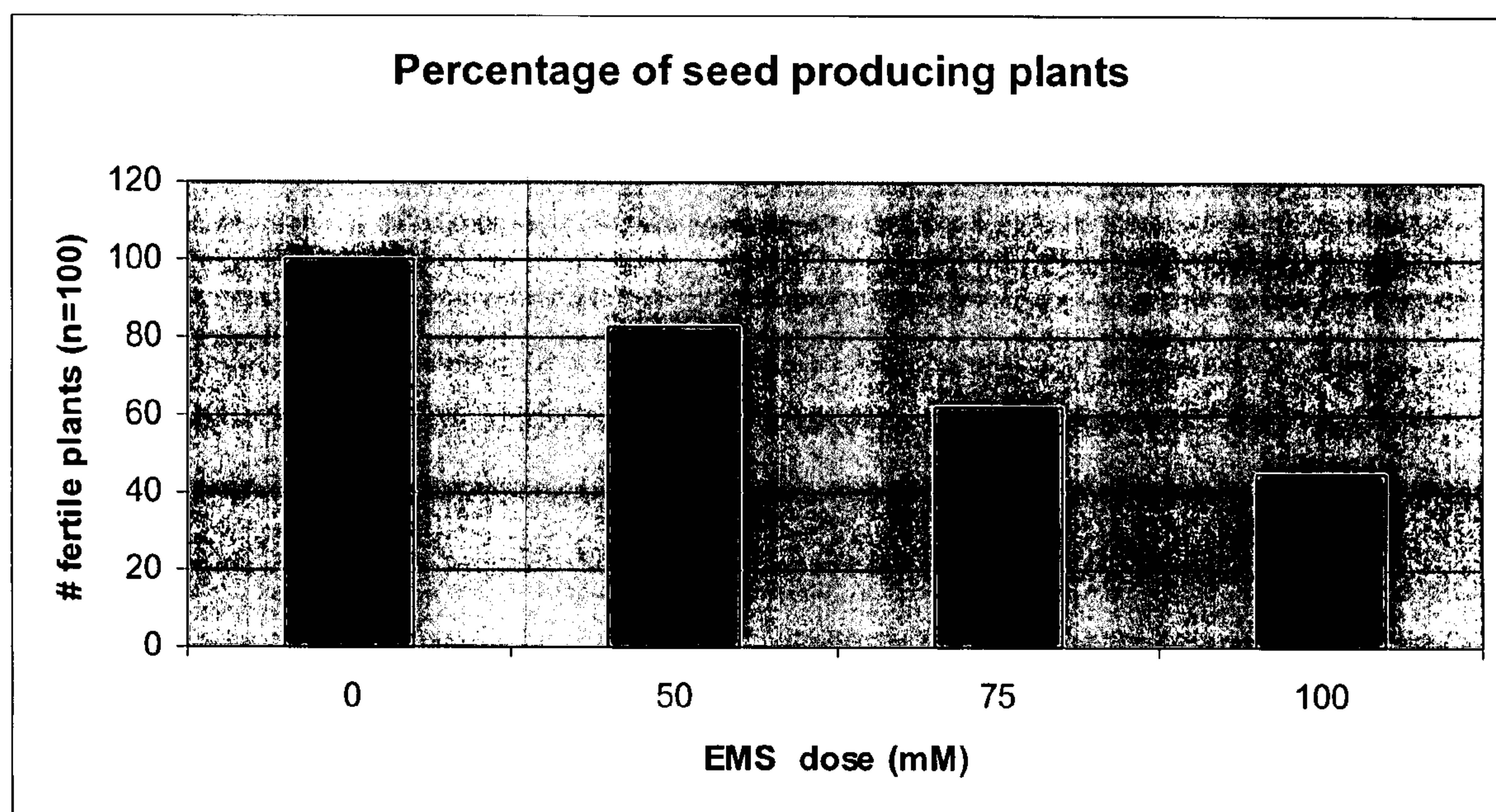


Figure 1B

XylTg14

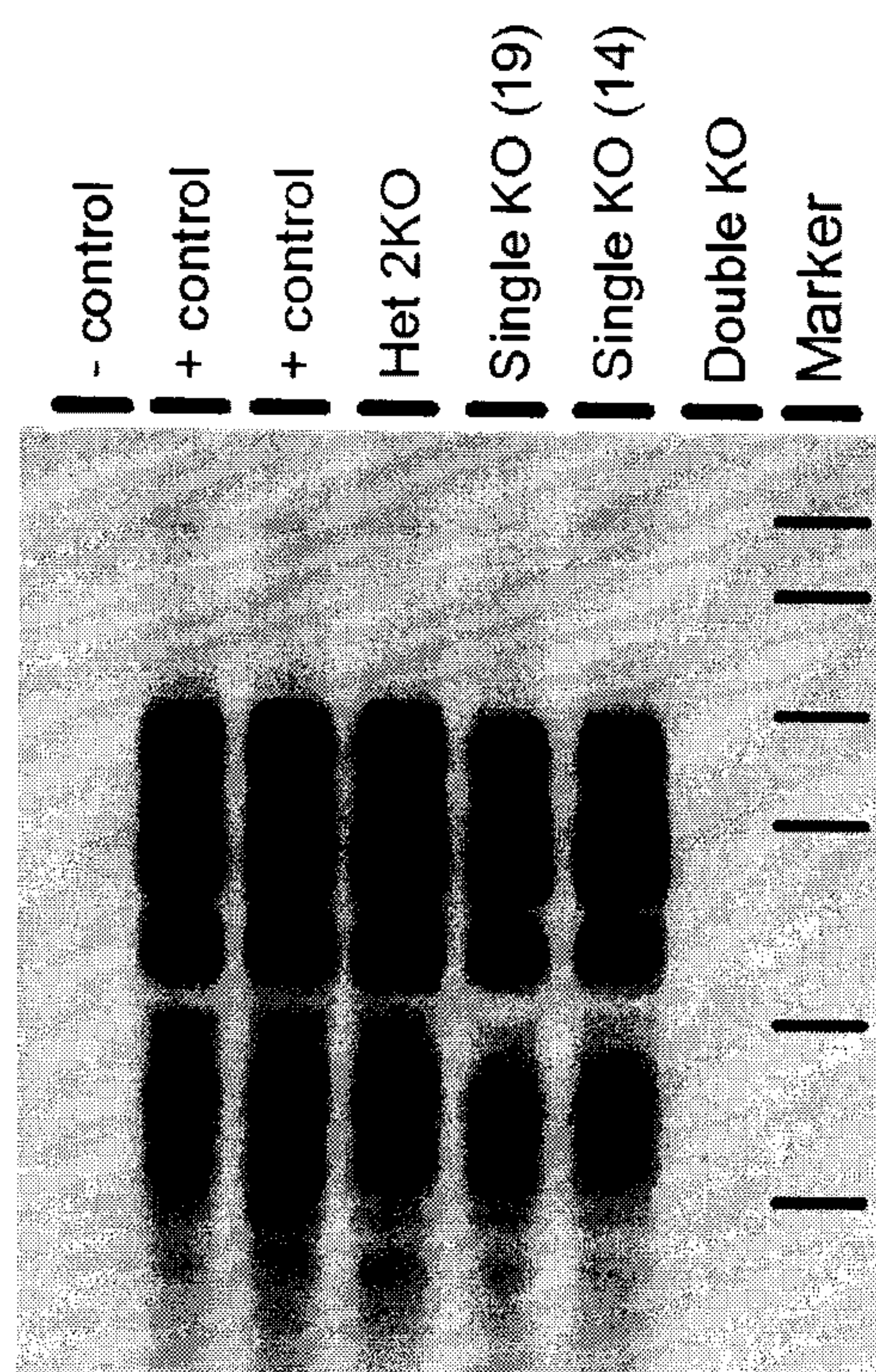
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										XylTg14-1
										T
101	GTCGGA AAAAC	CACCATCATA	ATTTCCACTC	TTCAATCACT	TCCCAATATT	CCAGGCCCTG	GCCATTTTGG	CCCTCCTACC	TCCCTTGGTC	TCAAAACCCCT
	CAGCCTTTTG	GTGGTAGTAT	TAAAGGTGAG	AAGITAGTGA	AGGGTTATAA	GGTCCGGAAC	CGGATAAAAC	GGGAGGATGG	AGGGAACCAG	AGTTTTGGGA
		XylTg14-2								NBNPGS2
		A								C
201	AATGTTGCTT	GGAGATCATG	CGAGGGTTAC	TTGSGTAATG	GTTTTACTCT	CAAAGTTGAT	CTTCTCAAAA	CTTCGCCGGA	GCTTCACCGG	AAATTCGGCG
	TTACAACGAA	CCTCTAGTAC	GCTCCCAATG	AAGCCATTAC	CAAAATGAGA	GTTTCAACTA	GAAGAGTTTT	GAAGCGGCCT	CGAAGTGGCC	TTTAAGCCCG
			XylTg14-3							
			A							
301	AAAACACCGT	CTTCGGAGAC	GGCGGATGGT	TTAGGTGTTT	CTTCAGTGAG	ACTTIGCAGA	GTTCGATCTG	CGAGGGAGGC	GCAATACGAA	TGAATCCAGA
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								NBNPGS2		
							C			
401	CGAGATTTTG	ATGTCTCGTG	GAGGTGAGAA	ATTGGAGTGG	GTTATTGGTA	GGAGTGAAGA	TGATGAGGTG	CCCSCGTTCA	AAACTGGAGC	TTTTCAGATT
	GCTCTAA AAC	TACAGAGCAC	CTCCACTCTT	TAACCTCAGC	CAATAACCAT	CCTCACTTCT	ACTACTCCAC	GGGCGCAAGT	TTTGACCTCG	AAAAGTCTAA
			NBNPGS2							
			T							
501	AAAGTTACTG	ATAAACTGAA	ATTTGCGAAA	AAATTAGTGG	ATCAAAAACCT	CTTGAATAAA	TACTTACCGG	AAGGTGCAAT	TTCAAGGCAC	ACTATGCGTG
	TTTCAATGAC	TATTTGACTT	TAAACCCCTT	TTTAATCACC	TACTTTTGAA	GAAC TTATTT	ATGAATGGCC	TTCCACGTTA	AAGTTCCGTG	TCATACGCAC
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	TCAATTAGCT	CAGATAAGTC	AACCAACCGC	GGTACTAAA	AGTGACAAGA	CTCCAATCTA	AAAAC TTAA	AACAAACGAG	AAATTTAATT	TCCAAACCTG
701	TTTGTGAATG	ITGGCAGATA	TAGAATACAA	TAATGSAATT	TGCTTGATCT	GTTTAATGAA	GATTGTCTGG	AACCTCAATG	CTATAAATAT	TTGTTTGT TT
	AAACACTTAC	AACCGTCTAT	ATCTTATGTT	ATTACCTTAA	ACGAAC TAGA	CAAA TTACTT	CTAACAGACC	TTGGAGTTAC	GATATTTATA	AACAAACAAA
801	GCTTCATTAA	TTAAAGAGAA	TATCCCAACT	AGATGCCAGA	TAACACCAGT	TAGTTGACTT	ITGGATCGGA	TTGCATTTCA	TTTAATCAGA	TATGGTACTC
	CGAAGTAATT	AATTTCTCTT	ATAGGGTTGA	TCTACGGTCT	ATTGTGGTCA	ATCAACTGAA	AACCTAGCCT	AACGTAAAGT	AAATTAGTCT	ATACCATGAG
901	ATTCTTAAAT	CTTTCACTAA	AGTATTTGTC	AAGATTTGAG	AGTTTATATG	TAGGTGTACT	TGGAAT TCTG	GATT TGGATC	IAGTATTGAA	TGGATTACTG
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1101	GTAAATGTGT	GTGGTCTAAG	ATGATTACTC	TAAC TTTTGA	GGTCTAACAT	GGAGAAAGTT	AGTTGATTTA	TGCTCTTTAC	TTTTCCCTTT	ATTGATTTTG
	CATTTACACA	CACCAGATTC	TACTAATGAG	ATTGAAAAC T	CCAGATTGTA	CCTCTTTCAA	TCAACTAAAT	ACGAGAAATG	AAAAGGAAA	TAACTAAAAC
1201	GCTTTTAAAT	TCTATCAATT	CCATTGTTTG	ATTGCTACTC	AAATTGAACC	TIAGACGGAG	TAGCAATAGC	AAAAAGTGAA	GAAAGGCCAT	TTTTTTTCTC
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1301	CTTTCATCTC	TTAATTTCCG	TTTTACACAC	AGAATATGGT	AGAA TCTGTT	TGAAGCTTTA	GTTGAATAGT	TATACAAC TG	GTTATTGCAT	TTTGAGGACT
	GAAAGTAGAG	AATTAAAGGC	AAAATGTGTG	TCTTATACCA	TCTTAGACAA	ACTTCGAAAT	CAACTTATCA	ATATGTTGAC	CAATAACGTA	AAACTCCTGA
1401	ATCGACTTGA	TTTGACACTG	GACAGTGTCT	CATACATGGC	TTGTAAGTTA	TGAGAAC TTC	TATCTAGGAA	GAAATCCCAA	CCAGAGATAG	GAGCTGATCA
	TAGCTGAACT	AAACTGTGAC	CTGTCA CAGA	CTATGTACCG	AACATTCAAT	ACTCTTGAAG	ATAGATCCTT	CTTTAGGGTT	GGTCTCTATC	CCTCGACAGT
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	GAACCGATAC	TCAATGACCG	AGTTTCAAGC	ICAAACTGGT	CAATTAAAAT	CTAGGAGTGG	TCTATTTGTA	AATCTCAGAT	IAGTTTAAGA	CTTCGTCATA
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1701	GGTCTTATAT	TTGCC TTTTA	CATTGCTCAT	GACTTGGGAT	ATTTCTGAAT	CAGCAATTTT	CAGTCTTTTA	TGTACTTATC	AAAAAATTAT	CCACTGCTAGA
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1801	IGTTAGTGTT	CAAGCAACCA	TGCTAGCATT	TAACGAAGCT	CCTTCTTTGA	TTCATGCGAT	CTTTCGGTAA	TCTATGCC TT	ACGTTACTGT	CATTTT TCTA
	ACAATCACAA	GTTCGTTGGT	ACGATCGTAA	ATTGCTTCGA	GGAAGAAACT	AAGTACGCTA	GAAAGGCATT	AGATACGGAA	TGCAATGACA	GTAAAAAGAT
1901	ATTTTCATTT	CAGTGGATTG	AGGAGCCGCT	ACTTTTGATT	ACACGATTTG	AGTATGCAAA	CCTTTTCCAC	ACAATTACCG	ATTGGTATAG	TGCATACGTG
	TAAAAGTAAA	GTCACTTAAC	TCCTCGGCAG	TGAAAAC TAA	TCTGTCTAAC	TCATACGTTT	GGAAAAGGTG	TGTTAATGGC	TAACCATATC	ACGTATGCAC
2001	GCATCGAGGG	TTACTGGCTT	GCCCACTCGG	CCACATTTGG	TTTTTGTAGA	TGGCCATTGT	GAGGTATGTC	TGAAAGTATT	GATAACGATG	GCATGCATTG
	CGTAGCTCCC	AATGACCGAA	CGGGTCAGCC	GGGTGTAACC	AAAAACATCT	ACCGGTAAACA	CTCCATACAG	ACTTTCATAA	CTATTGCTAC	CGTACGTAA C
2101	TACTGTCTTA	TGGATGAAAG	AAATGAAACC	AGCAATTATT	TTCTAGCAGG	CAATGCTCTT	GAGATGCTTG	TGTCAAATTG	GTCAAGCTTA	ATCCTGAGTT
	ATGACAGAA T	ACCTACTTTC	TTTACTTTGG	TCGTTAATAA	AAGATCGTCC	GTTACGAGAA	CTCTACGAAC	ACAGTTTAAC	CAGTCTGAA T	TAGGACTCAA
2201	TCCTATTGTT	TCAGCTTTCT	GTGTGACTGA	CTACAATTAAT	TGTCCCGATA	CCTAATTGTT	GCAGTTGGCT	CATTCTTATT	TCTATTTACG	TGTCACTGTT
	AGGTAAACAA	AGTCGAAAGA	CACACTGACT	GATGTTATTA	ACAGGCTAT	GGATTAACAA	CGTCAACCGA	GTAAGAATAA	AGATAAATGC	ACAGTGACAA
2301	TCTCTGAATG	GCCCTTTGTG	GTGAAAAGAG	CTTTTGTATAT	GTA AAAAAC	TAGCAAAAGAT	TTCA TTTCTG	GAACAATTTT	TTTTTACCTT	ACATCACGGG
	AGCAACTTAC	CGGGAACAC	CAC TTTTCTC	GAAAAC TATA	CATTTT TTTG	ATCGTTTCTA	AAGTAAAGAC	CTTGT TAAAG	AAAAATGGA A	TGTAGTGCAC
2401	TCATAAAATT	GCTTCTAACT	GTATACTTTA	ATTCTTGGAG	AGATGCTTTC	ATGTGAAGAA	AGTTCTTTCA	CTCCACTACT	GGAAGCTTGC	TGCATGAATT
	AGTATTTTAA	CGAAGATTGA	CATATGAAAT	TAAGAACCTC	TCTACGAAAG	TACACTTCTT	TCAAGAAAGT	GAGGTGATGA	CTTTCGAACG	ACGTACTTAA
2501	TTACTTGGCC	ATATTGGGGC	CGTGT TTTGA	TTTATCTTCA	AATTCATTTT	CTTCATGTAG	TTCTTTTCGAG	TAATTTTTTT	TCCTCTTTTC	TGTTTTGAAA
	AATGAACCGG	TATAACCCCG	GCACAAAAC T	AAATAGAAGT	TTAAGTAAAA	GAAGTACATC	AAGAAAGCTC	ATTA AAAAAA	AGGAGAAAAG	ACAAACTTTT
2601	AATTTCA GAC	ACAATTTGGAG	GAAACATGGA	AAGCATT TTT	TTCAAGCCTC	ACTTATGCTA	AGAACTTTAG	TGGCCCAGTT	TGTTTCCGTC	ATGCCGTCCT
	TTAAAGTCTG	TGTTAACCTC	CTTTGTACCT	TTGCTGAAAA	AAGTTCCGGAG	TGAATACGAT	TCTTGAAATC	ACCGGGTCAA	ACAAAGGCAG	TACGGCAGGA
2701	CTCGCCTTTG	CGATATGAAA	CTGCCCTGTT	TAAGGGACTG	TCAGAAACTA	TAGATTGTAA	TGGAGCTTCT	GCTCATGATT	TGTGGCAAAA	TCTGTATGAT
	GAGCGGAAAC	CCTATACTTT	GACGGGACAA	ATTCCCTGAC	AGTCTTTGAT	ATCTAACATT	ACCTCGAAGA	CGAGTACTAA	ACACCGTTTT	AGGACTACTA
2801	AAGAAAAC TG	CACGGTTATC	CGAGTTTGGG	GAGATGATCA	GGGCAGCCTT	TGGATTTCCT	GTTGATAGAC	AGAACATCCC	AAGGACAGTC	ACAGGCCCTA
	TTCTTTTGAC	GTGCCAATAG	GCTCAAACCC	CTCTACTAGT	CCCGTCGGAA	ACCTAAAGGA	CAACTATCTG	TCTTGTAGGG	TTCTGTCTAG	TGTCCGCGAT
2901	ATGCTCTCTT	TGTTAGACGT	GAGGATTATT	TAGCTACCCC	ACGTCTATGG	GGAAGGTATC	AGTCTAGGCT	TAGCAATGAA	GAGCAAGTAT	TTGATTCCAT
	TACAGGAGAA	ACAATCTGCA	CTCCTAATAA	ATCGAGTGGG	TGCAGTACCA	CCTTTCCATG	TCAGATCCGA	ATCGTTACTT	CTCGTTCATA	AAC TAAGGTA
3001	AAAGAGCTGG	GCCTTAAACC	ACTCGSAGTG	CAAA TTAAT	GTAATTAGTG	GATGT TTTG	CCACATGTCC	ATGAAAGAGC	AAGTTCCGAG	AATCCAGAT
	TTTTCTCGAC	CGGAATTTGG	TGAGCCTCAC	GT TTAATTTA	CATTAA TCA C	CTAACAAAAG	GGTGTACAGG	TACTTTCTCG	TTCAAGCTCG	TAGGTTCTA
3101	GCTTCTGTCA	TTGTTGGTGC	TCATGGAGCA	GCTCTAACCC	ACATAGTTTC	TGCAGCACCA	AAAGCTGTAA	TACTAGAAAT	TATAAGCAGC	GAAATATAGG
	CGAAGACAGT	AACAACCACG	AGTACCTCGT	CCAGATTGGG	TGATCAAAAG	ACGTCTGGGT	TTTCGACATT	ATGATCTTTA	ATATTCGTCG	CTTATATCCG
3201	CCCCCATTTT	TGCTCTGATT	GCTCAATGCA	AAGCATTCGA	GTACCATCCC	ATATATTTTG	AGGGCTCTTA	TGCGGATCCT	CCAGTTGTGA	TGGA
	CGCGGGTAAA	ACGAGACTAA	CCAGTTACCT	TTCTTAACCT	CATGGTAGGG	TATATAAACC	TCCCCAGAAT	ACGCCTAGGA	GGTCAACACT	AGCT

Figure 2A

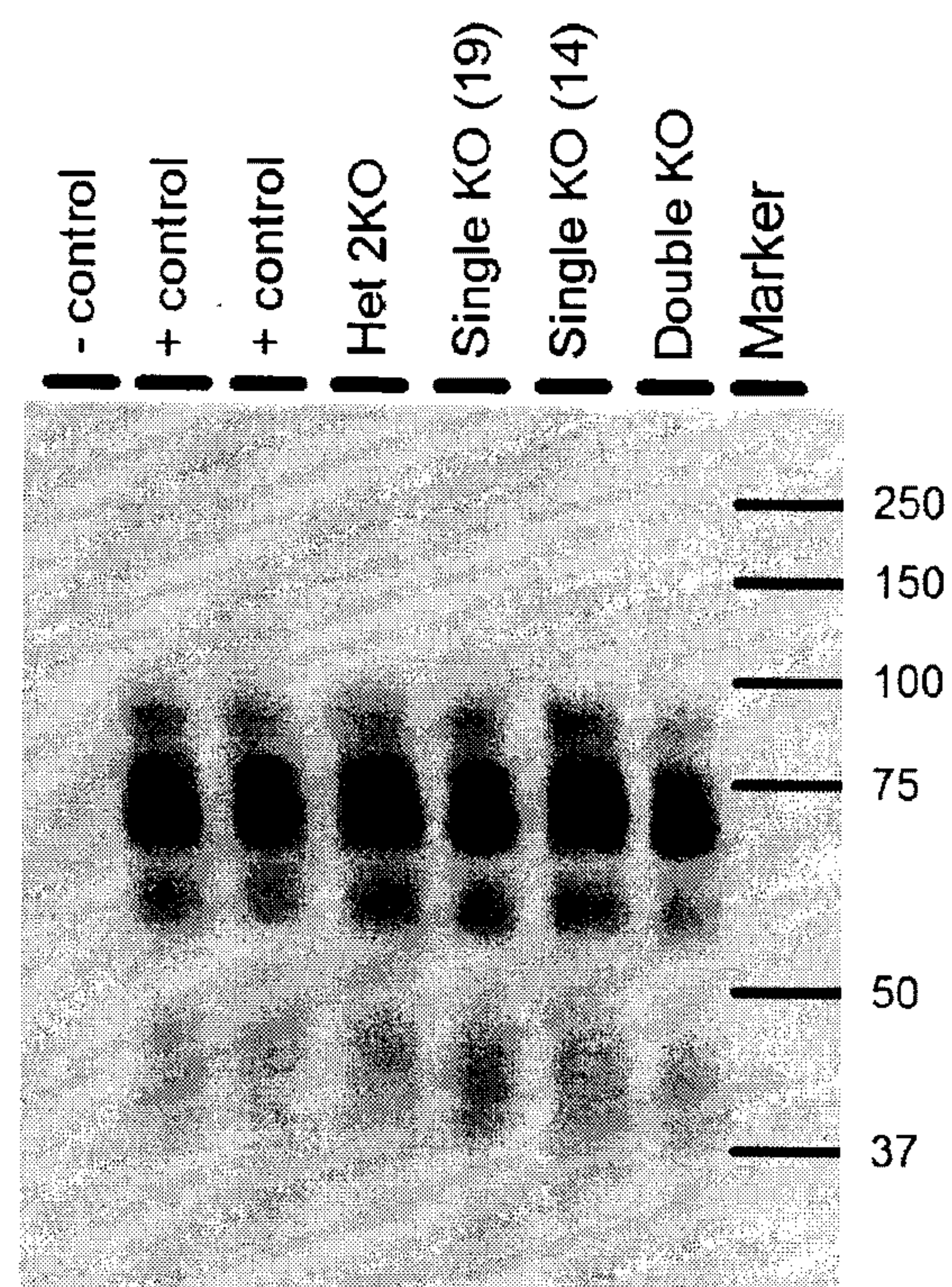
XylTg19

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	GTGGAACAAA	GAGAGAAGCG	AGAGTTGAGT	TAGTGACAGA	TAGAGATGAA	GAGAAGGGTG	GGACTACTGT	TTAGGGGGGT	TTTGGTGAAA	AGGAACAGCC
				XylTg19-1					XylTg19-2	
				T					A	
101	AAAACCACCA	TCATAATTTT	CACTCTTCAA	TCACTTCTCA	ATATTCCAAG	CCTTGCCCTA	TTTTGCCCTC	CTACCTCCCT	TGGTCTCAAA	ACCCTAATGT
	TTTTGGTGGT	AGTATTAAAG	GTGAGAAGTT	AGTGAAGAGT	TATAAGGTTC	GGAAACCGGAT	AAAACGGGAG	GATGGAG3GA	ACCAGAGTTT	TGGGATTACA
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	ACGAACCTCT	AGCACGCTCC	CAATGAAGCC	ATTACCCAAA	TGAGAGTTTC	AACCTGGAAGA	GTTTGAAGC	GGCCTCAAG	TGGCCTTTAA	GCCGCTATTG
									NBNPGS2	
									T	
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	TGGCAGAGGC	CACCTGCCGC	TACCAAATCC	ACAAAAAGT	CACCTCTGAAA	CCTCTCAAGC	TAGACGCTCC	CTCCGCGTTA	TCCTTACTTA	GGCCTGCTAT
401	TTTTGATGTC	TCGTGGAGGT	GAGAAATTGG	AGTCGGTTAT	TGGTAGGAAT	GAAGATGATG	AGCTGCCCAT	GTTCAAAAT	GGAGCTTTCC	AAATTGAAGT
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									NBNPGS2	
									A	
501	TACTGATAAA	CTGAAAATTG	GGAAAAAACT	AGTGGATAAA	AAATTCCTTGA	ATAAATACTT	ACCGGGAGGT	GCGATTTCAA	GGCACACTAT	GCGTGAGTTA
	ATGACTATTT	GACTTTTAACT	CCTTTTTTGA	TCACCTATTT	TTTAAGAAGT	TATTTATGAA	TGSCCCTCCA	CGCTAAAGTT	CCGTGTGATA	CGCACTCAAT
									NBNPGS2	
									T	
601	ATTGACTCTA	TTCAGTTGGT	TGGCGCCGAT	GAATTTCACT	GTCTGAGGT	TAGATTTTGA	TATTTATTTG	ATCTTTAAAT	TAGAGGTTTG	AACCTTTGTTA
	TAACCTGAGAT	AAGTCAACCA	ACCGCGGCTA	CTTAAAGTGA	CAAGACTCCA	ATCTAAAAC	ATAAATAAAC	TAGAAATTIA	ATCTCCAAAC	TTGAAACAAT
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	TACAACCGTC	TATACCTTAT	GTTATTACCT	AAAACAAACT	AGACAAATTA	CTTCTAACAG	ATTTTGGAGT	TACGATATTT	ATAACAAAC	AAACGAAGTA
801	TAATTAAGA	GAATATCCCG	ACTAGATGCC	AGATAACACC	AGTTAGTTGA	CTTTTGGATT	GGGTTGCATT	TCATTTAATC	AGATATGGTA	CTCATTTCTTA
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	TTACAAAGTG	ATTTCTTAAA	CAGTTCTAAA	GTCTCAATA	TATATCCACA	TAAACCTTAA	GACCTAAACC	TAGATCATAA	CTTACCTAAT	GACTTAAACA
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1201	TAAATTCAT	CAATTCCTTT	GTTTGCTTGC	TACTCAAATT	GAACCTTAGA	CGGAGTAGCA	ATAGCAAAAA	GTGAAGAAAG	GACATTTTTT	TCTCCTTCA
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1301	TCTCTTTATT	TCCGTTTGAC	ATACAGAATA	CGGTAGCATC	TGCTTGAAGT	GTTTAAATTC	ATTCCTTAAA	ATTTGCATAA	CTAATATTTT	CGTTTTTGT
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1401	TTTGTTTATC	TTTTCCATTG	GCAATGCCATG	TTATTTTTTG	TTTAGGTTTA	CATAATTATT	TATGTGATTT	CTGATGGAGT	TACTAATGAT	TTTTTGTTTT
	AAACAAATAG	AAAAGGTAACT	CGTACGGTAC	AATAAAAAACC	AAATCCAAAT	GTATTAAATA	ATACACTAAA	GACTACCTCA	ATGATTACTA	AAAAACAAA
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1801	GAGCTACTGG	CTTAAAGTTT	GAGTTTGACC	TATTAATTTT	AGATCTTCAC	CAGGATAACA	TCTAGAGTTT	AATTAATTC	TCAAGCAGTA	TTTTGCACTA
	CTCGATGACC	GAATTTCAAA	CTCAAAGTGG	ATAATTAATA	TGTAAAAGTG	GTCTAATGTT	ATGAATAGTT	TTAATTTAAG	AGTTCTGTCAT	AAACAGTGAT
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	TATTCCTCTT	GTGTACTTCC	TACATCGTGA	TGATGCAATA	CAAGAAATAA	ACGATAACTA	ACTGTTGGTC	GAATTTACTA	CTGTTTACCA	GAATATAAAC
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2201	GAGGAGCCGT	CACCTTTGAT	TACACGATT	GAGTATGCAA	ACCTTTTCCA	CACAGTTACC	GATTGTTATA	GTGCATACGC	GGCATCCAGG	GTTACTGGTT
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	CTTACTTTG	GTAGTTAATA	AAAGATCATG	CCTTACGAGA	ATTCACGAA	CAAGTTTAA	CCAATCTCAA	TTAGGATTCA	AAGGTAACAA	CACTCGAAG
2501	TGTTTGAAGT	ACTACAATAC	TTGTCCCAAT	ACCTAGTTGT	TGCGGTTGGC	TCATCTTAC	TTCTATTAC	GTGCTACTGT	TTCTCTGAAT	GGTCCCTTTG
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2601	TGGTGAAAAG	AGCTTTTGCT	ATGTAGAAAA	ACTAGCAAG	ATTTCAATTT	TGGAGCAACT	TATTTTACC	TTACATCACG	TCTCATAAAA	TTGCTTCTAA
	ACCACTCTTC	TCCAAAACGA	TACATCTTTT	TGATCGTTTC	TAAAGTAAAG	ACCTCGTTCA	ATAAAAAATG	AATGTAGTGC	AGAGTATTTT	AACCAAGATT
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	GACATATGAA	ATTAAGAACC	TCTCTACGAA	AGTACACTTA	TTTCAAGAAA	GTGAGGTGAT	GACCTTCGAA	CGACGTACTT	TAAATGAAC	GGTATGACCC
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	GCTCAAAACC	CTCTACTAGT	CCCGTCGGAA	ACCTAAAGGA	CACCTATCTG	TCTTGTAGGG	TTCTTGTGAG	TGTCGGGGAT	TACAGGAGAA	ACATCTTGCA
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	CTCCTAATAA	ATCGAGTGGG	TGCAGTACCA	CCTTTCCATG	TCAGATCCGA	ATCGTTACTT	CTCGATCATA	AACATAAGGTA	TTTCTCGACC	CGGAACCTGG
3301	ACTCCGAGTG	TAAATTAAAT	GTAATTAAAG	GATTGTTTGC	CCACATCTCC	ATGAAACAGC	AAGTTCCAGC	AATCCAAGAT	GCTTCTGTCA	TTGTTGGTGC
	TGAGCCTCAC	ATTTAATTTA	CATTAATTGC	CTAACAAACG	GGTGTACAGG	TACTTTCTCG	TTCAAGCTCG	TTAGGTTCTA	CGAAGACAGT	AACAACCAAG
3401	TGATGGAGCA	GCTCTAATCT	ACATAGTTTC	TGCAGCAGCA	AAAGCTGTAA	TACTAGAAAT	TATAAGCAGC	GAATATAGGC	GCCCCATTTT	TGCTCTGATT
	AGTACCTCGT	CCAGATTGAG	TGTATCAAAA	ACGTCGTGGT	TTTCGACATT	ATGATCTTTA	ATATTCTGTC	CTTATATCCG	CGGGGGTAAA	ACGAGACTAA
3501	GCACAATGGA	AAGGATTGGA	GTACCATCCC	ATATATTGGG	AGGGGTCTTA	TGCGGATCCT	CCAGTTGTGA	TCGA		
	GCTGTTACCT	TTCTTAACCT	CATGGTAGGG	TATATAAACC	TCCCCAGAAT	ACGCTTAGGA	GGTCAACACT	AGCT		

Figure 2B



Anti-xylose



Anti-fucose

Figure 3

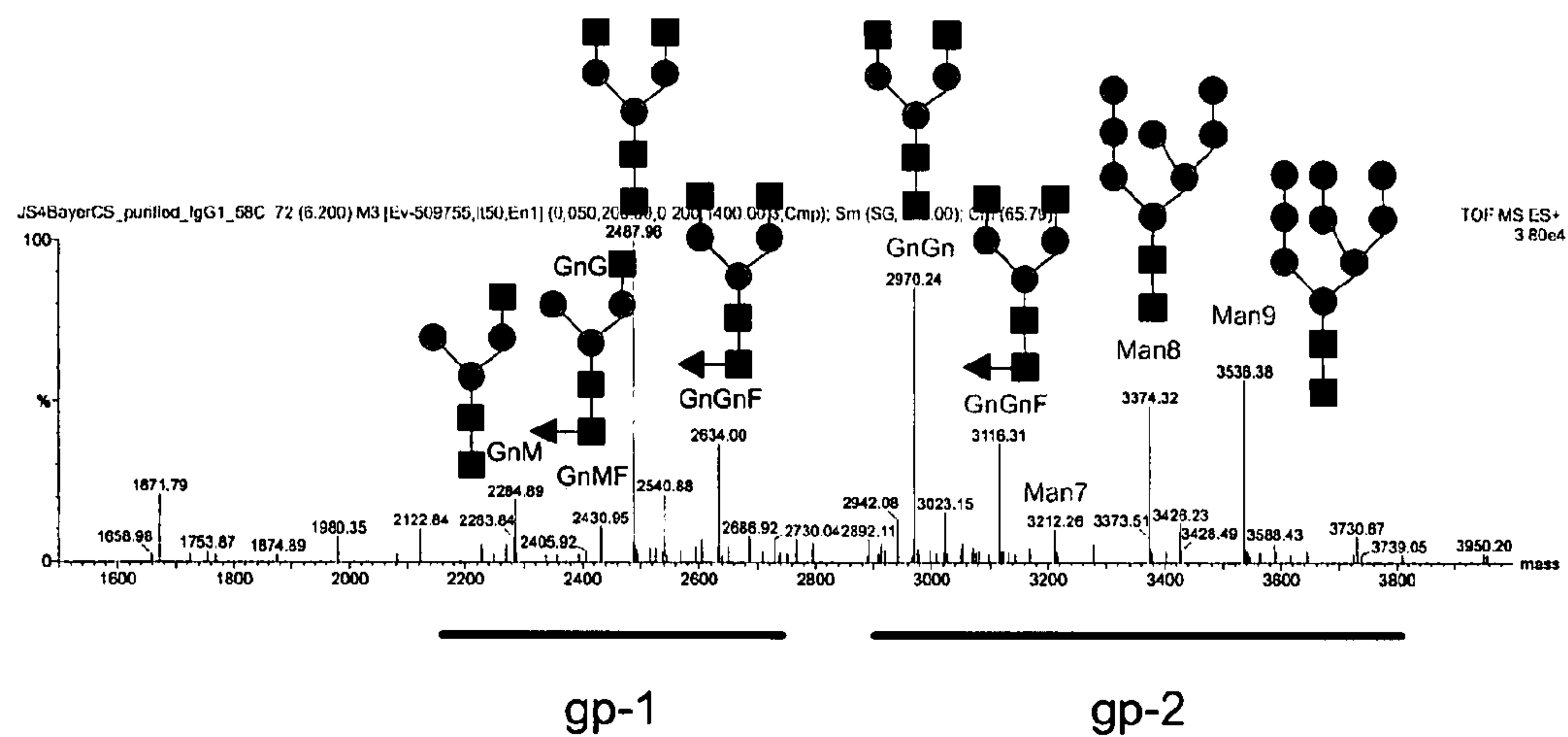


Figure 4

