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(54) **Title:** NOVEL NUCLEOTIDE SEQUENCES ENCODING *NICOTIANA* BETA-1,2-XYLOSYLTRANSFERASE

(57) **Abstract:** Provided are novelβ1,2-xylosyltransferase nucleotide sequences and uses thereof.

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Novel nucleotide sequences encoding *Nicotiana* beta-1,2-xylosyltransferase

The following invention relates to novel nucleotide sequences from *Nicotiana* species and cultivars, particularly from *Nicotiana benthamiana* and *Nicotiana tabacum* cv. Petite Havana SR1, encoding β1,2-xylosyltransferase (XylT) and their use to produce modified *Nicotiana* plants, particularly *Nicotiana benthamiana* and *Nicotiana tabacum* cv. Petite Havana SR1 plants, which have a lower level or altered pattern of immunogenic protein-bound N-glycans, particularly a lower level of beta-1,2-xylose residues on the protein-bound N-glycans, than counterpart unmodified *Nicotiana* plants. Such *Nicotiana* plants may be obtained by lowering the expression of the endogenous *Nicotiana* XylT gene(s), e.g., by modifying the activity of endogenous *Nicotiana* XylT gene(s), by exchanging the endogenous *Nicotiana* XylT gene for another allele of the XylT gene which provides a lower level of beta-1,2-xylose residues on the protein-bound N-glycans, or by any combination thereof.

Description of related art

The use of transgenic plants for the production of value-added recombinant proteins, such as antibodies, vaccines, human blood products, hormones, growth regulators and the like, is described to offer many practical, economic and safety advantages compared with more conventional systems such as animal and insect cell cultures, yeast, filamentous fungi and bacteria (reviewed by Stoger *et al.*, 2002; Twyman *et al.*, 2003; Fischer *et al.*, 2004).

Although the protein synthesis pathway is largely the same in plants and animals, there are some differences in posttranslational modifications, particularly with respect to glycan-chain structures. Thus, plant-derived recombinant human proteins tend to have the carbohydrate groups beta(1→2)-xylose and alpha(1→3)-fucose, which are absent in mammals, but lack the terminal galactose and sialic acid residues that are found on many native human glycoproteins (Twyman *et al.*, 2003).

The enzyme that catalyses the transfer of xylose from UDP-xylose to the core beta-linked mannose of protein-bound N-glycans is beta-1,2-xylosyltransferase (XylT). XylT is an enzyme

unique to plants and some non-vertebrate animal species, e.g. in *Schistosoma* species (Khoo *et al.*, 1997) and snail (e.g. Mulder *et al.*, 1995) and does not occur in human beings or in other vertebrates.

Tezuka *et al.* (1992) characterized a XylT of sycamore (*Acer pseudoplatanus* L.).

Zeng *et al.* (1997) described the purification of a XylT from soybean microsomes. Only a part of the soybean XylT cDNA was isolated (W099/29835 Al).

Strasser *et al.* (2000) and WO01/64901 describe the isolation of an *Arabidopsis* XylT gene, the predicted amino acid sequence of the encoded XylT protein and its enzymatic activity in vitro and *in vivo*.

The following database entries identifying experimentally demonstrated and putative XylT cDNA and gene sequences, parts thereof or homologous sequences, could be identified: AJ627182, AJ627183 (*Nicotiana tabacum* cv. Xanthi), AM179855 (*Solanum tuberosum*), AM179856 (*Vitis vinifera*), AJ891042 (*Populus alba* x *Populus tremula*), AY302251 (*Medicago sativa*), AJ864704 (*Saccharum officinarum*), AM179857 (*Zea mays*), AM179853 (*Hordeum vulgare*), AM179854 (*Sorghum bicolor*), BD434535, AJ277603, AJ272121, AF272852, AX236965 (*Arabidopsis thaliana*), AJ621918 (*Oryza sativa*), AR359783, AR359782, AR123000, AR123001 (Soybean), AJ618933 (*Physcomitrella patens*).

Strasser *et al.* (2004a) report on two approaches for the modulation of the N-glycosylation pathway in plants: First posttranscriptional gene silencing was used to knock down the expression of beta-1,2-N-acetylglucosaminyltransferase I (GnTI), an enzyme involved in the processing of oligomannosidic residues to hybrid and complex N-glycans in higher eukaryotes, to assess the influence of GnTI expression on the formation of complex N-glycans in *Nicotiana benthamiana*. N-glycan profiling revealed no significant changes of the total N-glycan pattern, indicating that even a minor residual activity of GnTI allows the biosynthesis of complex N-glycans in *Nicotiana benthamiana*. They further report that a similar approach for the knock down of XylT resulted in a significant reduction of beta-1,2-xylosylated N-glycans. Second, in

order to achieve a complete elimination of beta-1,2-xylose and alpha-1,3-fucose residues from N-glycans, triple knock out *Arabidopsis* plants were generated using insertion mutation lines. These plants exhibit complete deficiency of active beta-1,2-xylosyltransferase and core alpha-1,3-fucosyltransferase, lack immunogenic protein-bound N-glycans and synthesize predominantly humanized structures with terminal beta-N-acetylglucosamine residues (Strasser *et al.*, 2004b).

Leafy crops, such as tobacco, are considered to be strong candidates for the commercial production of recombinant proteins (see e.g. Twyman *et al.*, 2003).

The aim of the current invention is to provide alternative XylT cDNA and gene sequences from *Nicotiana* species and cultivars, particularly from *Nicotiana benthamiana* and *Nicotiana tabacum* cv. Petite Havana SR1, which are better suited to modify the expression of XylT in particular *Nicotiana* species or cultivars.

Summary of the invention

In one aspect of the invention, a method is provided to produce a *Nicotiana* plant cell or plant having a low level of beta-1,2-xylose residues on protein-bound N-glycans comprising the steps of introducing a chimeric gene into plant cells of a *Nicotiana* species or cultivar to generate transgenic plant cells, the chimeric gene comprising operably linked a plant expressible promoter; a transcribable DNA region comprising a first sense DNA region comprising a nucleotide sequence of at least 19 out of 20 consecutive nucleotides selected from a nucleotide sequence encoding a *Nicotiana* XylT protein, or the complement thereof, the nucleotide sequence preferably obtainable from the *Nicotiana* species or cultivar, wherein the at least 19 out of 20 consecutive nucleotides encode at least one *Nicotiana* species- or cultivar-specific XylT amino acid, or selected from a nucleotide sequence of a *Nicotiana* XylT gene or a *Nicotiana* XylT cDNA, or the complement thereof, the nucleotide sequence preferably obtainable from the *Nicotiana* species or cultivar, wherein the at least 19 out of 20 consecutive nucleotides comprise at least one *Nicotiana* species-specific XylT nucleotide; a second antisense DNA region comprising a nucleotide sequence of at least 19 consecutive nucleotides which have at least 95% sequence identity to the complement of the first DNA region; wherein an RNA molecule transcribed from the transcribable DNA region is capable of forming a double stranded RNA region at least between an RNA region transcribed from the first sense DNA region and an RNA region transcribed from the second antisense DNA region; and a DNA region comprising a transcription termination and polyadenylation signal functional in plants; optionally, identifying a transgenic *Nicotiana* plant cell which has a lower level of beta-1,2-xylose residues on protein-bound N-glycans than an untransformed *Nicotiana* plant cell; optionally, regenerating the transgenic *Nicotiana* plant cells to obtain transgenic *Nicotiana* plants; and optionally, identifying a transgenic *Nicotiana* plant which has a lower level of beta-1,2-xylose residues on protein-bound N-glycans than an untransformed *Nicotiana* plant. The *Nicotiana* species- or cultivar-specific XylT amino acid or nucleotide may be a *Nicotiana benthamiana*-specific or *Nicotiana tabacum* cv. Petite Havana SR1-specific XylT amino acid or nucleotide and the *Nicotiana* species or cultivar may preferably be *Nicotiana benthamiana* or *Nicotiana tabacum* cv. Petite Havana SR1, respectively. The nucleotide sequence encoding a *Nicotiana* XylT protein may comprise a nucleotide sequence encoding the amino acid

sequence of SEQ ID No.: 12 or SEQ ID No.:14 or the amino acid sequence of SEQ ID No.: 4, SEQ ID No.:6, SEQ ID No.: 8 or SEQ ID No.:10, and the nucleotide sequence of the *Nicotiana* XylT gene may comprise the nucleotide sequence of SEQ ID No.: 11, SEQ ID No.:13, or SEQ ID No. 21, or the nucleotide sequence of SEQ ID No.: 3, SEQ ID No.: 5, SEQ ID No.: 8, SEQ ID No.:10, or SEQ ID No.: 17.

It is another object of the invention to provide a method to produce a *Nicotiana* plant cell or plant having a low level of beta-1,2-xylose residues on protein-bound N-glycans comprising the steps of providing one or more double stranded RNA molecules to plant cells or plants of a *Nicotiana* species or cultivar, wherein the double stranded RNA molecules comprise two RNA strands, one RNA strand consisting essentially of an RNA nucleotide sequence of 19 out of 20 to 21 consecutive nucleotides selected from a nucleotide sequence encoding a *Nicotiana* XylT protein, or the complement thereof, the nucleotide sequence preferably obtainable from the *Nicotiana* species or cultivar, wherein the 19 out of 20 to 21 consecutive nucleotides encode at least one *Nicotiana* species- or cultivar-specific XylT amino acid, or selected from the nucleotide sequence of a *Nicotiana* XylT gene or a *Nicotiana* XylT cDNA, or the complement thereof, the nucleotide sequence preferably obtainable from the *Nicotiana* species or cultivar, wherein the 19 out of 20 to 21 consecutive nucleotides comprise at least one *Nicotiana* species- or cultivar-specific XylT nucleotide; and identifying a *Nicotiana* plant cell or plant comprising the double stranded RNA molecule or molecules which has a lower level of beta-1,2-xylose residues on protein-bound N-glycans than a same *Nicotiana* plant cell or plant which does not comprise the double stranded RNA molecule or molecules. The double stranded RNA may be provided to the plant cells or plants by integrating a chimeric gene into the genome of plant cells of the *Nicotiana* species or cultivar to generate transgenic plant cells and, optionally, regenerating the plant cells to obtain transgenic plants, the chimeric gene comprising a DNA region comprising at least 19 out of 20 consecutive nucleotides selected from a nucleotide sequence encoding a *Nicotiana* XylT protein, or the complement thereof, the nucleotide sequence preferably obtainable from the *Nicotiana* species or cultivar, wherein the 19 out of 20 consecutive nucleotides encode at least one *Nicotiana* species- or cultivar-specific XylT amino acid, or selected from the nucleotide sequence of a *Nicotiana* XylT gene or a *Nicotiana* XylT cDNA, or the complement thereof, the nucleotide sequence preferably obtainable from the

Nicotiana species or cultivar, wherein the 19 out of 20 consecutive nucleotides comprise at least one *Nicotiana* species-specific XylT nucleotide, in antisense and/or sense orientation; operably linked to a plant expressible promoter and a DNA region comprising a transcription termination and polyadenylation signal functional in plants. The *Nicotiana* species- or cultivar-specific XylT amino acid or nucleotide may be a *Nicotiana benthamiana*-specific or *Nicotiana tabacum* cv. Petite Havana SR1-specific XylT amino acid or nucleotide and the *Nicotiana* species or cultivar may preferably be *Nicotiana benthamiana* or *Nicotiana tabacum* cv. Petite Havana SR1, respectively. The nucleotide sequence encoding a *Nicotiana* XylT protein may comprise a nucleotide sequence encoding the amino acid sequence of SEQ ID No.: 12 or SEQ ID No.:14 or the amino acid sequence of SEQ ID No.: 4, SEQ ID No.:6, SEQ ID No.: 8 or SEQ ID No.:10, and the nucleotide sequence of the *Nicotiana* XylT gene may comprise the nucleotide sequence of SEQ ID No.: 11, SEQ ID No.:13, or SEQ ID No. 21, or the nucleotide sequence of SEQ ID No.: 3, SEQ ID No.: 5, SEQ ID No.: 8, SEQ ID No.:10, or SEQ ID No.: 17.

It is yet another object of the invention to provide a method to identify a *Nicotiana* XylT DNA fragment, comprising the steps of providing genomic DNA or cDNA obtainable from a *Nicotiana* species or cultivar; selecting a means from the following group: a DNA fragment comprising a nucleotide sequence encoding the amino acid sequence of SEQ ID No.: 4, SEQ ID No.:6, SEQ ID No.: 8, SEQ ID No.:10, SEQ ID No.: 12, or SEQ ID No.:14, for use as a probe; a DNA fragment comprising the nucleotide sequence of any one of SEQ ID No.: 3, SEQ ID No.: 5, SEQ ID No.: 7, SEQ ID No.: 9, SEQ ID No.: 11, SEQ ID No.: 13, SEQ ID No.: 17, or SEQ ID No.: 21, for use as a probe; a DNA fragment or oligonucleotide comprising a nucleotide sequence consisting of between 20 to 1513 consecutive nucleotides selected from a nucleotide sequence encoding the amino acid sequence of SEQ ID No.: 4, or SEQ ID No.:6, for use as a probe; a DNA fragment or oligonucleotide comprising a nucleotide sequence consisting of between 20 to 3574 consecutive nucleotides selected from a nucleotide sequence encoding the amino acid sequence of SEQ ID No.: 8, SEQ ID No.:10, SEQ ID No.: 12, or SEQ ID No.:14 for use as a probe; a DNA fragment or oligonucleotide comprising a nucleotide sequence consisting of between 20 to 3574 consecutive nucleotides selected from a nucleotide sequence of any one of SEQ ID No.: 3, SEQ ID No.: 5, SEQ ID No.: 7, SEQ ID No.: 9, SEQ

ID No.: 11, SEQ ID No.: 13, SEQ ID No.: 17, or SEQ ID No.: 21 for use as a probe; an oligonucleotide sequence having a nucleotide sequence comprising between 20 to 200 consecutive nucleotides selected from a nucleotide sequence encoding the amino acid sequence of SEQ ID No.: 4, or SEQ ID No.:6, for use as a primer in a PCR reaction; an oligonucleotide sequence having a nucleotide sequence comprising between 20 to 200 consecutive nucleotides selected from a nucleotide sequence encoding the amino acid sequence of SEQ ID No.: 8, SEQ ID No.:10, SEQ ID No.: 12, or SEQ ID No.:14 , for use as a primer in a PCR reaction; an oligonucleotide sequence having a nucleotide sequence comprising between 20 to 200 consecutive nucleotides selected from the nucleotide sequence of any one of SEQ ID No.: 3, SEQ ID No.: 5, SEQ ID No.: 7, SEQ ID No.: 9, SEQ ID No.: 11, SEQ ID No.: 13, SEQ ID No.: 17, or SEQ ID No.: 21, for use as a primer in a PCR reaction; or an oligonucleotide having the nucleotide sequence of any one of SEQ ID No.: 1, SEQ ID No.: 2, SEQ ID No.: 15 or SEQ ID No.: 16, SEQ ID No.:19 or SEQ ID No.20 for use as a primer in a PCR reaction; and utilizing that means to identify a XylT DNA fragment from the *Nicotiana* species or cultivar by performing a PCR using the genomic DNA or the cDNA and the primers, or by performing hybridization using the genomic DNA or the cDNA and the probes. The identified fragment may subsequently be isolated and used to obtain a *Nicotiana* plant cell or plant having a low level of beta-1,2-xylose residues on protein-bound N-glycans.

The invention also provides a method to identify a *Nicotiana* XylT allele correlated with a low level of beta-1,2-xylose residues on protein-bound N-glycans comprising the steps of providing a population, optionally a mutagenized population, of different plant lines of a *Nicotiana* species or cultivar; identifying in each plant line of the population a *Nicotiana* XylT allele according to the method described above; analyzing the level of beta-1,2-xylose residues on protein-bound N-glycans of each plant line of the population and identifying those plant lines having a lower level of beta-1,2-xylose residues on protein-bound N-glycans than other plant lines; and correlating the low level of beta-1,2-xylose residues on protein-bound N-glycans in a plant line to the presence of a specific *Nicotiana* XylT allele. The *Nicotiana* XylT allele may be introduced into a *Nicotiana* plant cell or plant of choice to obtain a *Nicotiana* plant cell or plant with a low level of beta-1,2-xylose residues on protein-bound N-glycans.

It is yet another object of the invention to provide: an isolated DNA fragment encoding a protein comprising the amino acid sequence of SEQ ID No.: 12, or SEQ ID No.:14, or any part thereof encoding at least one *Nicotiana benthamiana*-specific XylT amino acid; an isolated DNA fragment comprising the nucleotide sequence of SEQ ID No.: 11, SEQ ID No.: 13, or SEQ ID No.: 21, or any part thereof comprising at least one *Nicotiana benthamiana*-specific XylT nucleotide; an isolated DNA fragment encoding a protein comprising the amino acid sequence of SEQ ID No.: 4 or SEQ ID No.:6, SEQ ID No.: 8, SEQ ID No.:10, or any part thereof encoding at least one *Nicotiana tabacum* cv. Petite Havana SR1-specfic XylT amino acid; an isolated DNA fragment comprising the nucleotide sequence of SEQ ID No.: 3 or SEQ ID No.:5, SEQ ID No.: 7, SEQ ID No.:9, or SEQ ID No.: 17, or any part thereof comprising at least one *Nicotiana tabacum* cv. Petite Havana SR1-specfic XylT nucleotide.

The invention further provides a chimeric gene comprising the following operably linked DNA fragments: a plant expressible promoter; a transcribable DNA region comprising a first DNA region comprising at least 19 out of 20 consecutive nucleotides selected from a nucleotide sequence encoding a *Nicotiana* XylT protein, or the complement thereof, wherein the 19 out of 20 consecutive nucleotides encode at least one *Nicotiana* species- or cultivar-specific XylT amino acid, or selected from the nucleotide sequence of a *Nicotiana* XylT gene or a *Nicotiana* XylT cDNA, or the complement thereof, wherein the 19 out of 20 consecutive nucleotides comprise at least one *Nicotiana* species-specific XylT nucleotide, in antisense orientation; a second DNA region comprising at least 19 out of 20 consecutive nucleotides selected from a nucleotide sequence encoding a *Nicotiana* XylT protein, or the complement thereof, wherein the 19 out of 20 consecutive nucleotides encode at least one *Nicotiana* species- or cultivar-specific XylT amino acid, or selected from the nucleotide sequence of a *Nicotiana* XylT gene or a *Nicotiana* XylT cDNA, or the complement thereof, wherein the 19 out of 20 consecutive nucleotides comprise at least one *Nicotiana* species-specific XylT nucleotide, in sense orientation, whereby an RNA molecule produced by transcription of the transcribable DNA region is capable of forming a double stranded RNA region by base-pairing at least between an RNA region corresponding to the first DNA region and an RNA region corresponding to the second DNA region; and a DNA region comprising a transcription termination and polyadenylation signal functional in plants. The chimeric gene may also comprise a plant

expressible promoter; a DNA region comprising at least 19 out of 20 consecutive nucleotides selected from a nucleotide sequence encoding a *Nicotiana* XylT protein, or the complement thereof, wherein the 19 out of 20 consecutive nucleotides encode at least one *Nicotiana* species- or cultivar-specific XylT amino acid, or selected from the nucleotide sequence of a *Nicotiana* XylT gene or a *Nicotiana* XylT cDNA, or the complement thereof, wherein the 19 out of 20 consecutive nucleotides comprise at least one *Nicotiana* species-specific XylT nucleotide, in sense or antisense orientation; and a DNA region comprising a transcription termination and polyadenylation signal functional in plants.

Nicotiana plant cells comprising such chimeric genes and *Nicotiana* plants consisting essentially of such *Nicotiana* plant cells, as well as seed thereof are also provided by the invention.

The invention also relates to the use of a nucleotide sequence encoding a protein comprising the amino acid sequence of SEQ ID No.: 4, SEQ ID No.:6, SEQ ID No.: 8, SEQ ID No.:10, SEQ ID No.: 12, or SEQ ID No.:14, or any part thereof comprising at least 19 out of 20 consecutive nucleotides encoding at least one *Nicotiana* species- or cultivar-specific XylT amino acid, to decrease the level of beta-1,2-xylose residues on protein-bound N-glycans in a *Nicotiana* plant, or the use of a nucleotide sequence comprising the nucleotide sequence of SEQ ID No.: 3, SEQ ID No.:5, SEQ ID No.: 7, SEQ ID No.:9, SEQ ID No.: 11, SEQ ID No.: 13, SEQ ID No.: 17 or SEQ ID No.: 21, or any part thereof comprising at least 19 out of 20 consecutive nucleotides comprising at least one *Nicotiana* species- or cultivar-specific XylT nucleotide, to decrease the level of beta-1,2-xylose residues on protein-bound N-glycans in a *Nicotiana* plant, to identify a XylT gene or XylT cDNA in a *Nicotiana* species or cultivar, to identify an allele of a *XylT* gene correlated with a low level of beta-1,2-xylose residues on protein-bound N-glycans in a *Nicotiana* species or cultivar, or to introduce an allele of a *XylT* gene correlated with a low level of beta-1,2-xylose residues on protein-bound N-glycans in a *Nicotiana* species or cultivar.

With the foregoing and other objects, advantages and features of the invention that will become hereinafter apparent, the nature of the invention may be more clearly understood by reference

to the following detailed description of different embodiments of the invention, the appended claims and the figures.

Brief description of the Figures

Figure 1 is a global DNA alignment (based on the standard linear scoring matrix with following parameters: mismatch penalty = 2, open gap penalty = 4 and extend gap penalty = 1) between a cDNA sequence of *Nicotiana tabacum* cv. Xanthi encoding a putative XylT protein (accession number AJ627182; SEQ ID NO:23) and two different XylT cDNA sequences isolated from *Nicotiana tabacum* cv. Petite Havana SR1 (SEQ ID NO: 3 and 5). Dots represent nucleotides in the *Nicotiana tabacum* cv. Petite Havana SR1 cDNA sequences that are identical to the corresponding nucleotides in the *Nicotiana tabacum* cv. Xanthi cDNA sequence; dashes represent the absence of nucleotides in the *Nicotiana tabacum* cv. Petite Havana SR1 cDNA sequences corresponding to nucleotides in the *Nicotiana tabacum* cv. Xanthi cDNA sequence.

Figure 2 is a global protein alignment (based on the blossum 62 scoring matrix) between the putative XylT protein encoded by the cDNA sequence from *Nicotiana tabacum* cv. Xanthi (accession number AJ627182; SEQ ID NO:24) and by the two different XylT cDNA sequences isolated from *Nicotiana tabacum* cv. Petite Havana SR1 (SEQ ID NO: 4 and 6). Dots represent amino acids in the *Nicotiana tabacum* cv. Petite Havana SR1 protein sequences that are identical to the corresponding amino acids in the *Nicotiana tabacum* cv. Xanthi protein sequence; dashes represent the absence of amino acids in the *Nicotiana tabacum* cv. Petite Havana SR1 protein sequences corresponding to amino acids in the *Nicotiana tabacum* cv. Xanthi protein sequence.

Figure 3 is a global DNA alignment (based on the standard linear scoring matrix with following parameters: mismatch penalty = 2, open gap penalty = 4 and extend gap penalty = 1) between the genomic DNA sequence from *Nicotiana tabacum* cv. Xanthi encoding a putative XylT protein (accession number AJ627183; SEQ ID NO:25) and two different XylT genomic DNA sequences isolated from *Nicotiana tabacum* cv. Petite Havana SR1 (SEQ ID NO: 7 and 9) and two different XylT genomic DNA sequences isolated from *Nicotiana benthamiana* (SEQ ID NO: 11 and 13). Dots represent nucleotides in the *Nicotiana tabacum* cv. Petite Havana SR1 genomic DNA sequences that are identical to the corresponding nucleotides in the

Nicotiana tabacum cv. Xanthi genomic DNA sequence; dashes represent the absence of nucleotides in the *Nicotiana tabacum* cv. Petite Havana SR1 genomic DNA sequences corresponding to nucleotides in the *Nicotiana tabacum* cv. Xanthi genomic DNA sequence.

Figure 4 is a global protein alignment (based on the blossum 62 scoring matrix) between the putative XylT protein encoded by the genomic DNA sequence from *Nicotiana tabacum* cv. Xanthi (accession number AJ627183; SEQ ID NO:26) and by the two different XylT genomic DNA sequences isolated from *Nicotiana tabacum* cv. Petite Havana SR1 (SEQ ID NO:8 and 10) and by the two different XylT genomic DNA sequences isolated from *Nicotiana benthamiana* (SEQ ID NO: 12 and 14). Dots represent amino acids in the *Nicotiana tabacum* cv. Petite Havana SR1 protein sequences that are identical to the corresponding amino acids in the *Nicotiana tabacum* cv. Xanthi protein sequence; dashes represent the absence of amino acids in the *Nicotiana tabacum* cv. Petite Havana SR1 protein sequences corresponding to amino acids in the *Nicotiana tabacum* cv. Xanthi protein sequence.

Detailed description of different embodiments of the invention

The current invention is based on the finding that XylT genes and XylT cDNAs from *Nicotiana* species and cultivars, particularly *Nicotiana benthamiana* and *Nicotiana tabacum* cv. Petite Havana SR1, are excellent source nucleotide sequences to obtain plants of those *Nicotiana* species and cultivars, particularly *Nicotiana benthamiana* plants and *Nicotiana tabacum* cv. Petite Havana SR1 plants, respectively, having a low level of beta-1,2-xylose residues on protein-bound N-glycans, e.g., by modifying the activity of endogenous *Nicotiana* XylT gene(s), by exchanging an endogenous *Nicotiana* XylT gene for another allele of the *Nicotiana* XylT gene which provides a low level of beta-1,2-xylose residues on protein-bound N-glycans, or by any combination thereof.

In one embodiment, the invention is related to a method for obtaining a *Nicotiana* plant cell or plant having a low level of beta-1,2-xylose residues on protein-bound N-glycans by reducing the expression of the endogenous XylT gene(s) in the *Nicotiana* plant cell or plant by providing one or more silencing RNA molecules to plant cells or plants of a *Nicotiana* species or cultivar, wherein the silencing RNA molecules comprise a part of a nucleotide sequence encoding a *Nicotiana* XylT protein, preferably obtained from said *Nicotiana* species or cultivar, wherein said part encodes at least one *Nicotiana* species- or cultivar-specific XylT amino acid, or wherein the silencing RNA molecules comprise a part of a nucleotide sequence of a *Nicotiana* XylT gene or a *Nicotiana* XylT cDNA, preferably obtained from said *Nicotiana* species or cultivar, wherein said part comprises at least one *Nicotiana* species- or cultivar-specific XylT nucleotide.

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 (both documents herein incorporated by reference). Yet another type of silencing RNA is an RNA molecule as described in WO03/076619 (herein incorporated by reference) comprising at least 20 consecutive nucleotides having 95% sequence identity to the sequence of the target nucleic acid or the complement thereof, and further 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 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 region form a double stranded RNA region. hpRNA is well-known within the art (see e.g WO99/53050, herein incorporated by reference). 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 in size from about 30 to about 42 bp, but not much longer than 94 bp (see WO04/073390, herein incorporated by reference). Silencing RNA may also be artificial micro-RNA molecules as described e.g. in WO2005/052170, WO2005/047505 or US 2005/0144667 (all documents incorporated herein by reference)

In another embodiment, the silencing RNA molecules are provided to the plant cell or plant of the *Nicotiana* species or cultivar by producing a transgenic plant cell or plant of the *Nicotiana* species or cultivar comprising a chimeric gene capable of producing a silencing RNA molecule, particularly a double stranded RNA ("dsRNA") molecule, wherein the complementary RNA strands of such a dsRNA molecule comprises a part of a nucleotide

sequence encoding a *Nicotiana* XylT protein, preferably obtained from said *Nicotiana* species or cultivar, wherein said part encodes at least one *Nicotiana* species- or cultivar-specific XylT amino acid, or wherein the complementary RNA strands of such a dsRNA molecule comprises a part of the nucleotide sequence of a *Nicotiana* XylT gene or a *Nicotiana* XylT cDNA, preferably obtained from said *Nicotiana* species or cultivar, wherein said part comprises at least one *Nicotiana* species- or cultivar-specific XylT nucleotide.

"*Nicotiana*", as used herein, includes all known *Nicotiana* species, such as, but not limited to, *Nicotiana acaulis, N. acuminata, N. africana, N. alata, N. amplexicaulis, N. arentsii, N. attenuata, N. benavidesii, N. benthamiana, N. bigelovii, N. bonariensis, N. cavicola, N. clevelandii, N. cordifolia, N. corymbosa, N. debneyi, N. excelsior, N. forgetiana, N. fragrans, N. glauca, N. glutinosa, N. goodspeedii, N. gossei, N. hybrid, N. ingulba, N. kawakamii, N. knightiana, N. langsdorffii, N. linearis, N. longiflora, N. maritima, N. megalosiphon, N. miersii, N. noctiflora, N. nudicaulis, N. obtusifolia, N. occidentalis, N. otophora, N. paniculata, N. pauciflora, N. petunioides, N. plumbaginifolia, N. quadrivalvis, N. raimondii, N. repanda, N. rosulata, N. rotundifolia, N. rustica, N. setchellii, N. simulans, N. solanifolia, N. spegazzinii, N. stocktonii, N. suaveolens, N. sylvestris, N. tabacum, N. thyrsiflora, N. tomentosa, N. tomentosiformis, N. trigonophylla, N. umbratica, N. undulata, N. velutina, N. wigandioides, and Nicotiana x sandera,* and all known *Nicotiana* cultivars, such as, but not limited to, cultivars of *Nicotiana tabacum*, such as cv. Burley21, cv. Delgold, cv. Petit Havana, cv. Petit Havana SR1, cv. Samsun, and cv. Xanthi.

Nicotiana tabacum, which is common tobacco, is a tetraploid hybrid species, which originated from the diploid species *Nicotiana sylvestris* and *Nicotiana tomentosiformis*.

A *Nicotiana* XylT gene or a *Nicotiana* XylT cDNA refers to a nucleotide sequence of a XylT gene that naturally occurs in a *Nicotiana* species or cultivar or to cDNA corresponding to the mRNA of a XylT gene that naturally occurs in a *Nicotiana* species or cultivar. Similarly, a *Nicotiana* XylT protein refers to a protein as it naturally occurs in a *Nicotiana* species or cultivar.

Examples of nucleotide sequences encoding a *Nicotiana* XylT protein, include those obtained from *Nicotiana benthamiana* encoding the amino acid sequence set forth in SEQ ID No.: 12 or SEQ ID No.: 14, and those obtained from *Nicotiana tabacum* cv. Petite Havana SR1 encoding the amino acid sequence set forth in SEQ ID No.: 4, SEQ ID No.:6, SEQ ID No.: 8, or SEQ ID No.:10.

Examples of nucleotide sequences of a *Nicotiana* XylT gene include those obtained from *Nicotiana benthamiana* comprising the nucleotide sequence set forth in SEQ ID No.: 11, SEQ ID No.: 13, or SEQ ID No.: 21, and those obtained from *Nicotiana tabacum* cv. Petite Havana SR1 comprising the nucleotide sequence set forth in SEQ ID No.: 7 or SEQ ID No.: 9.

Examples of nucleotide sequences of a *Nicotiana* XylT cDNA, include those obtained from *Nicotiana tabacum* cv. Petite Havana SR1 comprising the nucleotide sequence set forth in SEQ ID No.: 3, SEQ ID No.: 5 or SEQ ID No.: 17.

However, it will be immediately clear to the person skilled in the art that the exemplified nucleotide sequences or parts thereof can be used to identify further nucleotide sequences of *Nicotiana* XylT genes or *Nicotiana* XylT cDNAs in *Nicotiana* species or cultivars, and that such nucleotide sequences or parts thereof may also be used e.g. to decrease the level of beta-1,2-xylose residues on protein-bound N-glycans in *Nicotiana* plants. The exemplified nucleotide sequences could be used to select:

i) a DNA fragment comprising a nucleotide sequence encoding the amino acid sequence of SEQ ID No.: 4, SEQ ID No.:6, SEQ ID No.: 8, SEQ ID No.:10, SEQ ID No.: 12, or SEQ ID No.:14, for use as a probe;

ii) a DNA fragment comprising the nucleotide sequence of any one of SEQ ID No.: 3, SEQ ID No.: 5, SEQ ID No.: 7, SEQ ID No.: 9, SEQ ID No.: 11, SEQ ID No.: 13, SEQ ID No.: 17, or SEQ ID No.: 21, for use as a probe;

iii) a DNA fragment or oligonucleotide comprising a nucleotide sequence consisting of between 20 to 1513 consecutive nucleotides selected from a nucleotide sequence encoding the amino acid sequence of SEQ ID No.: 4, or SEQ ID No.:6, for use as a probe;

iv) a DNA fragment or oligonucleotide comprising a nucleotide sequence consisting of between 20 to 3574 consecutive nucleotides selected from a nucleotide sequence encoding the amino acid sequence of SEQ ID No.: 8, SEQ ID No.:10, SEQ ID No.: 12, or SEQ ID No.:14 , for use as a probe

v) a DNA fragment or oligonucleotide comprising a nucleotide sequence consisting of between 20 to 3574 consecutive nucleotides selected from a nucleotide sequence of any one of SEQ ID No.: 3, SEQ ID No.: 5, SEQ ID No.: 7, SEQ ID No.: 9, SEQ ID No.: 11, SEQ ID No.: 13, SEQ ID No.: 17, or SEQ ID No.: 21, for use as a probe;

vi) an oligonucleotide sequence having a nucleotide sequence comprising between 20 to 200 consecutive nucleotides selected from a nucleotide sequence encoding the amino acid sequence of SEQ ID No.: 4, or SEQ ID No.:6, for use as a primer in a PCR reaction;

vii) an oligonucleotide sequence having a nucleotide sequence comprising between 20 to 200 consecutive nucleotides selected from a nucleotide sequence encoding the amino acid sequence of SEQ ID No.: 8, SEQ ID No.:10, SEQ ID No.: 12, or SEQ ID No.:14 , for use as a primer in a PCR reaction;

viii) an oligonucleotide sequence having a nucleotide sequence comprising between 20 to 200 consecutive nucleotides selected from the nucleotide sequence of any one of SEQ ID No.: 3, SEQ ID No.: 5, SEQ ID No.: 7, SEQ ID No.: 9, SEQ ID No.: 11, SEQ ID No.: 13, SEQ ID No.: 17, or SEQ ID No.: 21, for use as a primer in a PCR reaction; or

ix) an oligonucleotide having the nucleotide sequence of any one of SEQ ID No.: 1, SEQ ID No.: 2, SEQ ID No.: 15 or SEQ ID No.: 16, SEQ ID No.:19 or SEQ ID No.20 for use as a primer in a PCR reaction.

By performing a PCR using genomic DNA or cDNA from *Nicotiana* species or cultivars and the mentioned oligonucleotides as primers or by performing hybridization, preferably under stringent conditions between genomic or cDNA from *Nicotiana* species or cultivars and the mentioned probes, such other *Nicotiana* XylT genes or *Nicotiana* XylT cDNAs or fragments thereof can be identified and/or isolated.

"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 μg/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, e.g. for about 10 min (twice). 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.

A "*Nicotiana* species-specific XylT nucleotide" or a "*Nicotiana* cultivar-specific XylT nucleotide", refers to a nucleotide of the nucleotide sequence of a XylT gene or a XylT cDNA from a *Nicotiana* species or cultivar that differs from or is not present in the corresponding nucleotide sequence of the XylT gene from *Nicotiana tabacum* cv. Xanthi represented in SEQ ID NO: 25, or of the XylT cDNA from *Nicotiana tabacum* cv. Xanthi represented in SEQ ID NO: 23, respectively.

A "*Nicotiana* species-specific XylT amino acid" or a "*Nicotiana* cultivar-specific XylT amino acid", refers to an amino acid of the amino acid sequence of a XylT protein encoded by a XylT gene or encoded by a XylT cDNA from a *Nicotiana* species or cultivar that differs from or is not present in the corresponding amino acid sequence of the XylT protein encoded by the XylT gene from *Nicotiana tabacum* cv. Xanthi represented in SEQ ID NO: 26, or encoded by the XylT cDNA from *Nicotiana tabacum* cv. Xanthi represented in SEQ ID NO: 24, respectively.

To determine the presence of a *Nicotiana* species- or cultivar-specific XylT nucleotide or amino acid in the nucleotide sequence of a XylT gene or a XylT cDNA from a *Nicotiana* species or cultivar or in the amino acid sequence of a XylT protein encoded by a XylT gene or encoded by a XylT cDNA from a *Nicotiana* species or cultivar, for the purpose of this invention, the XylT nucleotide or amino acid sequences from the *Nicotiana* species or cultivar

are compared with the corresponding XylT nucleotide or amino acid sequences from *Nicotiana tabacum* cv. Xanthi by aligning the sequences using a global alignment procedure (For nucleotide sequences the default scoring matrix used is "standard linear" with mismatch penalty = 2, open gap penalty = 4 and extend gap penalty = 1. For protein sequences the default scoring matrix is "blosum 62"; Henikoff and Henikoff, 1992.). To perform the alignment the Align Plus program (provided by Scientific & Educational Software, USA) may be used.

One example of such a global DNA alignment is the global DNA alignment of the XylT cDNA sequence from *Nicotiana tabacum* cv. Xanthi represented in SEQ ID NO:23 with the XylT cDNA sequences from *Nicotiana tabacum* cv. Petite Havana SR1 represented in SEQ ID NO:3 and 5, in Figure 1. Examples of *Nicotiana tabacum* cv. Petite Havana SR1–specific XylT nucleotides determined based on this global DNA alignment include:

- the nucleotide at position 1041, 1323, 1332, or 1421 of SEQ ID NO:3,
- the nucleotide at position 62, 76, 87, 104, 117, 122, 139, 140, 148, 155, 169, 190, 199, 202, 212, 213, 216, 265, 287, 294, 316, 373, 385, 388, 430, 554, 607, 628, 643, 838, 892, 897, 898, 941, 1005, 1021, 1039, or 1495 of SEQ ID NO:5.

Another example of such a global DNA alignment is the global DNA alignment of the XylT gene sequence from *Nicotiana tabacum* cv. Xanthi represented in SEQ ID NO:25 with the XylT gene sequences from *Nicotiana tabacum* cv. Petite Havana SR1 represented in SEQ ID NO:7 and 9 and with the XylT gene sequences from *Nicotiana benthamiana* represented in SEQ ID NO:11 and 13, in Figure 3. Examples of *Nicotiana tabacum* cv. Petite Havana SR1–specific XylT nucleotides determined based on this global DNA alignment include:

- the nucleotide at position 61, 75, 86, 100-120, 124, 137, 142, 159, 160, 168, 175, 189, 210, 219, 222, 232, 233, 236, 285, 307, 314, 336, 393, 405, 408, 450, 574, 627, 648, 663, 692, 698, 702, 721, 754, 802, 821, 842, 852, 856, 901, 903, 906, 907, 908, 917, 927, 928, 930, 931, 960, 961, 965, 974, 977, 981, 983, 986, 1001, 1019, 1027, 1029, 1034, 1068, 1073, 1099, 1120, 1129, 1144, 1154, 1158, 1181, 1193, 1208, 1212, 1228, 1230, 1239, 1275, 1313-1316, 1348, 1353, 1357, 1384, 1386, 1496, 1531, 1571, 1601, 1629, 1681, 1696, 1698, 1730, 1754, 1761, 1772, 1789, 1800, 1802, 1811, 1814, 1815, 1855-1861, 1929, 2172, 2190, 2322, 2324, 2328, 2353, 2354, 2391, 2404, 2419, 2428,

2429, 2433, 2434, 2459, 2464, 2478, 2479, 2481, 2484, 2512, 2540-2590, 2595, 2604, 2606, 2630, 2633, 2638, 2670, 2673, 2680, 2695, 2698, 2710, 2711, 2752, 2806, 2811, 2812, 2855, 2919, 2953, 2966, 3217, 3226, 3229, or 3232 of SEQ ID NO:7,

- the nucleotide at position 553, 606, 627, 642, 671, 677, 681, 700, 733, 781, 800, 821, 831, 835, 880, 882, 885, 886, 887, 896, 906, 907, 909, 910, 939, 940, 944, 953, 956, 960, 962, 965, 980, 998, 1006, 1008, 1013, 1047, 1052, 1078, 1099, 1108, 1123, 1133, 1137, 1160, 1172, 1187, 1191, 1207, 1209, 1218, 1254, 1292, 1293, 1294, 1295, 1327, 1332, 1336, 1363, 1365, 1475, 1510, 1550, 1580, 1608, 1660, 1675, 1677, 1709, 1733, 1740, 1751, 1768, 1779, 1781, 1790, 1793, 1794, 1834-1840, 1908, 2151, 2169, 2301, 2303, 2307, 2332, 2333, 2370, 2383, 2398, 2407, 2408, 2412, 2413, 2438, 2443, 2457, 2458, 2460, 2463, 2491, 2519-2569, 2574, 2583, 2585, 2609, 2612, 2617, 2649, 2652, 2659, 2674, 2677, 2689, 2690, 2731, 2785, 2790, 2791, 2834, 2898, 2932, 2945, 3196, or 3205 of SEQ ID NO:9

Examples of *Nicotiana benthamiana*–specific XylT nucleotides determined based on this global DNA alignment include:

- the nucleotide at position 71, 72, 75, 77, 86, 90, 104, 116, 147, 158, 212, 222, 246, 264, 286, 317, 321, 345, 402, 472, 479, 488, 526, 552, 612, 637, 668, 669, 670, 701, 726, 734, 742, 747, 773, 785, 795, 796, 802, 831, 871, 872, 874, 888, 897, 898, 899, 901, 902, 927, 931, 932, 1039, 1044, 1047, 1080, 1091, 1103, 1104, 1113, 1118, 1131, 1134, 1145, 1152, 1164, 1179, 1183, 1199, 1201, 1206, 1227, 1286, 1287, 1288, 1289, 1296, 1301, 1317, 1328, 1332, 1347, 1376, 1388, 1424, 1429, 1458, 1464, 1510, 1517, 1534, 1559, 1672, 1675, 1676, 1677, 1693, 1705, 1719, 1750, 1757, 1761, 1765, 1832, 1838, 1862, 1872, 1877, 1978, 2010, 2074, 2111, 2115, 2227, 2251, 2259, 2271, 2283, 2296, 2297, 2341, 2348, 2361, 2370, 2371, 2375, 2384, 2401, 2404, 2406, 2495, 2497, 2521, 2529, 2561, 2607, 2701, 2777, 2822, 2843, 2856, 2867, 3020, 3052, 3053, 3116, 3143, or 3227 of SEQ ID NO:11
- the nucleotide at position 77, 107, 203, 297, 312, 399, 449, 469, 489, 492, 496, 529, 538, 566, 573, 633, 661, 662, 666, 671, 683, 690, 699, 723, 763, 774, 784, 785, 791, 861, 877, 886, 887, 888, 890, 891, 920, 921, 943, 996, 1015, 1034, 1116, 1190, 1226, 1277-1280, 1282, 1287, 1331, 1343, 1360, 1386-1651, 1672, 1689, 1738, 1770, 1791, 1813, 1820, 1822, 1831, 1832, 1862, 1869, 1874, 1882, 1893, 1906, 1935, 1945, 1956,

1988, 2007, 2033, 2034, 2045, 2049, 2050, 2167, 2198, 2280, 2299, 2315, 2355, 2392, 2413, 2428, 2442, 2464, 2468, 2477, 2493, 2522, 2544, 2548, 2573, 2574, 2639, 2648, 2649, 2653, 2655, 2659, 2679, 2684, 2740, 2773, 2775, 2781, 2796, 2799, 2807, 2816, 2839, 2857, 2975, 2977, 2990, 3053, 3071, 3083, 3119, 3132, 3265, 3311, or 3392 of SEQ ID NO:13.

One example of such a global protein alignment is the global protein alignment of the XylT protein sequence encoded by the XylT cDNA sequence from *Nicotiana tabacum* cv. Xanthi represented in SEQ ID NO:24 with the XylT protein sequences encoded by the XylT cDNA sequences from *Nicotiana tabacum* cv. Petite Havana SR1 represented in SEQ ID NO:4 and 6, in Figure 2. Examples of *Nicotiana tabacum* cv. Petite Havana SR1–specific XylT amino acids determined based on this global protein alignment include:

- the amino acid at position 472 or 502 of SEQ ID NO:4,
- the amino acid at position 20, 28, 38, 40, 46, 51, 70, 71, 95, 97, 184, 213, 298, 313, 334, or 497 of SEQ ID NO:6.

Another example of such a global protein alignment is the global protein alignment of the XylT protein sequences encoded by the XylT gene sequence from *Nicotiana tabacum* cv. Xanthi represented in SEQ ID NO:26 with the XylT protein sequences encoded by the XylT gene sequences from *Nicotiana tabacum* cv. Petite Havana SR1 represented in SEQ ID NO:8 and 10 and with the XylT protein sequences encoded by the XylT gene sequences from *Nicotiana benthamiana* represented in SEQ ID NO:12 and 14, in Figure 4. Examples of *Nicotiana tabacum* cv. Petite Havana SR1–specific XylT amino acids determined based on this global protein alignment include:

- the amino acid at position 19, 27, 32-38, 44, 46, 52, 57, 76, 77, 101, 103, 190, 219, 304, 319, 340, or 356 of SEQ ID NO:8,
- the amino acid at position 183, 212, 297, 312, 333, or 349 of SEQ ID NO:10.

Examples of *Nicotiana benthamiana*–specific XylT amino acids determined based on this global protein alignment include:

- the amino acid at position 22, 24, 27, 33, 37, 51, 69, 94, 104, 156, 158, 161, 174, 182, 211, 238, 297, 349, or 414 of SEQ ID NO:12,

- the amino acid at position 1, 26, 36, 68, 99, 133, 150, 157, 166, 180, 189, 211, 218, 245, 257, 296, 327, 348, or 392 of SEQ ID NO:14.

The part of the nucleotide sequence encoding a *Nicotiana* XylT protein and the part of the nucleotide sequence of a *Nicotiana* XylT gene or a *Nicotiana* XylT cDNA comprised within the silencing RNA molecule, particularly within one strand of the double stranded RNA molecule, should be at least 19 nucleotides long, but may vary from about 19 nucleotides (nt) up to a length equalling the length (in nucleotides) of the *Nicotiana* XylT protein-encoding sequence or the *Nicotiana* XylT gene or cDNA sequence. The total length of the sense or antisense nucleotide sequence may thus be at least 25 nt, or at least about 50 nt, or at least about 100 nt, or at least about 150 nt, or at least about 200 nt, or at least about 500 nt. It is expected that there is no upper limit to the total length of the sense or the antisense nucleotide sequence. However for practical reason (such as e.g. stability of the chimeric genes) it is expected that the length of the sense or antisense nucleotide sequence should not exceed 5000 nt, particularly should not exceed 2500 nt and could be limited to about 1000 nt.

It will be appreciated that the longer the total length of the part of nucleotide sequence encoding a *Nicotiana* XylT protein or the part of the nucleotide sequence of a *Nicotiana* XylT gene or a *Nicotiana* XylT cDNA (sense or antisense region), the less stringent the requirements for sequence identity between these regions and the corresponding sequence in the endogenous XylT gene from the *Nicotiana* species or cultivar it complements are. Preferably, the nucleic acid of interest should have a sequence identity of at least about 75% with the corresponding target sequence, particularly at least about 80 %, more particularly at least about 85%, quite particularly about 90%, especially about 95%, more especially about 100%, quite especially be identical to the corresponding part of the target sequence or its complement. However, it is preferred that the nucleic acid of interest always includes a sequence of about 19 consecutive nucleotides, particularly about 25 nt, more particularly about 50 nt, especially about 100 nt, quite especially about 150 nt with 100% sequence identity to the corresponding part of the target XylT nucleic acid, wherein said about 19 consecutive nucleotides, particularly about 25 nt, more particularly about 50 nt, especially about 100 nt, quite especially about 150 nt, encode

at least one *Nicotiana* species- or cultivar-specific XylT amino acid or comprise at least one *Nicotiana* species- or cultivar-specific XylT nucleotide.

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. Preferably, for calculating the sequence identity and designing the corresponding sense or antisense sequence, the number of gaps should be minimized, particularly for the shorter sense sequences.

It will be clear that whenever nucleotide sequences of RNA molecules are defined by reference to nucleotide sequence of corresponding DNA molecules, the thymine (T) in the nucleotide sequence should be replaced by uracil (U). Whether reference is made to RNA or DNA molecules will be clear from the context of the application.

It has been demonstrated that the minimum requirement for silencing a particular target gene is the presence in the silencing chimeric gene nucleotide sequence of a nucleotide sequence of about 20-21 consecutive nucleotides long corresponding to the target gene sequence, in which at least 19 of the 20-21 consecutive nucleotides are identical to the corresponding target gene sequence. "19 out of 20 consecutive nucleotides" as used herein refers to a nucleotide sequence of 20 consecutive nucleotides selected from the target gene having one mismatch nucleotide.

For silencing the endogenous XylT gene from a *Nicotiana* species or cultivar, it is preferred that the silencing chimeric gene nucleotide sequence comprises at least 19 out of 20-21 consecutive nucleotides from a nucleotide sequence encoding a *Nicotiana* XylT protein, wherein said at least 19 out of 20-21 consecutive nucleotides encode at least one *Nicotiana* species- or cultivar-specific XylT amino acid, or comprises at least 19 out of 20-21 consecutive nucleotides from a nucleotide sequence of a *Nicotiana* XylT gene or a *Nicotiana* XylT cDNA,

wherein said at least 19 out of 20-21 consecutive nucleotides comprise at least one *Nicotiana* species- or cultivar-specific XylT nucleotide.

As used herein "a *Nicotiana* plant having a low level of beta-1,2-xylose residues on protein-bound N-glycans" is a plant (particularly a *Nicotiana* plant obtained according to the methods of the invention), in which the XylT activity is decreased or abolished resulting in a lower level of beta-1,2-xylose residues on protein-bound N-glycans than the level of beta-1,2-xylose residues on protein-bound N-glycans in a control *Nicotiana* plant not treated according to the methods of the invention or resulting in the absence of beta-1,2-xylose residues on protein-bound N-glycans. An indication of XylT activity can be obtained by comparing the level of beta-1,2-xylose residues present on the glycans of proteins from the *Nicotiana* plant obtained according to the methods of the invention with the level of beta-1,2-xylose residues present on the glycans of proteins from a control *Nicotiana* plant not treated according to the methods of the invention. The level of beta-1,2-xylose residues on protein-bound N-glycans of plants can be measured e.g. by Western blot analysis using xylose-specific antibodies as described e.g. by Faye *et al.* (Analytical Biochemistry, 1993, 209: 104-108) or by mass spectrometry on glycans isolated from the plant's glycoproteins using Matrix-assisted Laser Desorption/Ionization Time-of-Flight mass spectronomy (MALDI-TOF-MS) as described e.g. by Kolarich and Altmann (2000, Anal. Biochem. 285: 64-75) or using Liquid Chromatography Tandem mass spectronomy (LC/MS/MS) as described e.g. by Henriksson *et al.* (2003, Biochem. J. 375: 61–73).

dsRNA encoding *Nicotiana* XylT expression reducing chimeric genes according to the invention may comprise an intron, such as a heterologous intron, located e.g. in the spacer sequence between the sense and antisense RNA regions in accordance with the disclosure of WO 99/53050 (incorporated herein by reference).

It has become apparent that double stranded RNA molecules, such as the ones described above, are cleaved in plant cells into small RNA fragments of about 20-21 nucleotides, which serve as guide sequence in the degeneration of the corresponding mRNA (reviewed by Baulcombe, 2004). Thus, in another embodiment, the invention is drawn to a method for producing a

Nicotiana plant cell or plant having a low level of beta-1,2-xylose residues on protein-bound N-glycans comprising the steps of:

a) providing one or more double stranded RNA molecules to cells of a plant of a *Nicotiana* species or cultivar, wherein the double stranded RNA molecules comprise two RNA strands, one RNA strand consisting essentially of an RNA nucleotide sequence of 20 to 21 consecutive nucleotides selected from a nucleotide sequence encoding a *Nicotiana* XylT protein, preferably obtained from said *Nicotiana* species or cultivar, wherein said 20 to 21 consecutive nucleotides encode at least one *Nicotiana* species- or cultivar-specific XylT amino acid, or one RNA strand consisting essentially of an RNA nucleotide sequence of 20 to 21 consecutive nucleotides from a nucleotide sequence of a *Nicotiana* XylT gene or a *Nicotiana* XylT cDNA, preferably obtained from said *Nicotiana* species or cultivar, wherein said 20 to 21 consecutive nucleotides comprise at least one *Nicotiana* species- or cultivar-specific XylT nucleotide; and

b) identifying a *Nicotiana* plant comprising these double stranded RNA molecule or molecules which has a lower level of beta-1,2-xylose residues on protein-bound N-glycans than a same *Nicotiana* plant which does not comprise the double stranded RNA molecule or molecules.

The mentioned 20-21 nt long dsRNA sequences are also generated in the course of conventional antisense RNA mediated silencing or sense RNA mediated silencing. Thus, in another embodiment of the invention, a method is provided for producing a *Nicotiana* plant cell or plant having a low level of beta-1,2-xylose residues on protein-bound N-glycans, comprising the step of providing to cells of a plant of the *Nicotiana* species or cultivar a chimeric gene comprising, operably linked, the following DNA fragments

a) a plant expressible promoter;

b) a DNA region comprising at least 20 consecutive nucleotides selected from a nucleotide sequence encoding a *Nicotiana* XylT protein, preferably obtained from said *Nicotiana* species or cultivar, wherein said at least 20 consecutive nucleotides encode at least one *Nicotiana* species- or cultivar-specific XylT amino acid, or comprising at least 20 consecutive nucleotides from a nucleotide sequence of a *Nicotiana* XylT gene or a *Nicotiana* XylT cDNA, preferably obtained from said *Nicotiana* species or cultivar,

wherein said at least 20 consecutive nucleotides comprise at least one *Nicotiana* species- or cultivar-specific XylT nucleotide, in antisense or in sense orientation;

c) a DNA region comprising a transcription termination and polyadenylation signal functional in plants.

The mentioned antisense or sense nucleotide regions may thus be from about 21 nt to about 5000 nt long, such as 21nt, 40 nt, 50 nt, 100nt, 200 nt, 300nt, 500nt, 1000 nt, or even about 2000 nt or larger in length. Moreover, it is not required for the purpose of the invention that the nucleotide sequence of the used inhibitory XylT gene molecule or the encoding region of the chimeric gene, is completely identical or complementary to the endogenous *Nicotiana* XylT gene the expression of which is targeted to be reduced in the *Nicotiana* plant cell. The longer the sequence, the less stringent the requirement for the overall sequence identity is. Thus, the sense or antisense regions may have an overall sequence identity of about 40 % or 50% or 60 % or 70% or 80% or 90 % or 100% to the nucleotide sequence of the endogenous *Nicotiana* gene or the complement thereof. However, as mentioned, antisense or sense regions should preferably comprise a nucleotide sequence of 19-20 consecutive nucleotides having about 100% sequence identity to the nucleotide sequence of the XylT gene, wherein said 19-20 consecutive nucleotides, encode at least one *Nicotiana* species- or cultivar-specific XylT amino acid or comprise at least one *Nicotiana* species- or cultivar-specific XylT nucleotide. The stretch of about 100 % sequence identity may be about 50, 75 or 100 nt.

The efficiency of the above mentioned chimeric genes which when transcribed yield antisense or sense silencing RNA may be further enhanced by inclusion of DNA elements which result in the expression of aberrant, unpolyadenylated XylT inhibitory RNA molecules. One such DNA element suitable for that purpose is a DNA region encoding a self-splicing ribozyme, as described in WO 00/01133. The efficiency may also be enhanced by providing the generated RNA molecules with nuclear localization or retention signals as described in WO 03/076619.

The exemplified XylT nucleotide sequences from *Nicotiana benthamiana* and from *Nicotiana tabacum* can also be used to identify XylT alleles in a population of plants of a *Nicotiana* species or cultivar which are correlated with low levels of beta-1,2-xylose residues on protein-

bound N-glycans. Such populations of plants of a *Nicotiana* species or cultivar may be populations which have been previously mutagenized. The identified XylT alleles may then be introduced into a plant line of a *Nicotiana* species or cultivar of choice using conventional breeding techniques.

Methods to transform *Nicotiana* plants are also well known in the art. *Agrobacterium*-mediated transformation of *Nicotiana* has been described e.g. in Zambryski *et al.* (1983, EMBO J. 2: 2143–2150), De Block *et al.* (1984, EMBO J. 3(8):1681-1689), or Horsch *et al.* (Science (1985) 227: 1229-1231).

The obtained transformed *Nicotiana* plants according to the invention, or the obtained *Nicotiana* plants having a low level of beta-1,2-xylose residues on protein-bound N-glycans wherein the endogenous XylT gene has been replaced by a XylT allele, which is correlated with a lower levels of beta-1,2-xylose residues on protein-bound N-glycans than the original XylT allele, can be used in a conventional breeding scheme to produce more plants with the same characteristics or to introduce the chimeric gene according to the invention in other cultivars of the same or related plant species, or in hybrid plants. Seeds obtained from the transformed plants contain the chimeric genes of the invention as a stable genomic insert and are also encompassed by the invention.

Furthermore, it is known that introduction of antisense, sense or doublestranded RNA or the encoding chimeric genes may lead to a distribution of phenotypes, ranging from almost no or very little suppresion of the expression of the target gene to a very strong or even a 100% suppression of the expression of the target gene. However, a person skilled in the art will be able to select those plant cells, plants, events or plant lines leading to the desired degree of silencing and desired phenotype.

As used herein "comprising" is to be interpreted as specifying the presence of the stated features, integers, steps or components as referred to, but does not preclude the presence or addition of one or more features, integers, steps or components, or groups thereof. Thus, e.g., a nucleic acid or protein comprising a sequence of nucleotides or amino acids, may comprise

more nucleotides or amino acids than the actually cited ones, i.e., be embedded in a larger nucleic acid or protein. A chimeric gene comprising a DNA region, which is functionally or structurally defined, may comprise additional DNA regions etc.

The following non-limiting Examples describe chimeric genes for the alteration of the level of beta-1,2-xylose residues on protein-bound N-glycans in *Nicotiana* species, particularly in *Nicotiana benthamiana*, and in *Nicotiana* cultivars, particularly in *Nicotiana tabacum* cv. Petite Havana SR1, and uses thereof. Unless stated otherwise in the Examples, all recombinant DNA techniques are carried out according to standard protocols as described in Sambrook *et al.* (1989) Molecular Cloning: A Laboratory Manual, Second Edition, Cold Spring Harbor Laboratory Press, NY and in Volumes 1 and 2 of Ausubel *et al.* (1994) Current Protocols in Molecular Biology, Current Protocols, USA. Standard materials and methods for plant molecular work are described in Plant Molecular Biology Labfax (1993) by R.D.D. Croy, jointly published by BIOS Scientific Publications Ltd (UK) and Blackwell Scientific Publications, UK.

Throughout the description and Examples, reference is made to the following sequences represented in the sequence listing:

SEQ ID NO: 1: nucleotide sequence of the oligonucleotide XylF4 suitable to amplify a part of a *Nicotiana* XylT gene or cDNA.

SEQ ID NO: 2: nucleotide sequence of the oligonucleotide XylR4 suitable to amplify a part of a *Nicotiana* XylT gene or cDNA.

SEQ ID NO: 3: partial cDNA sequence of *Nicotiana tabacum* cv. Petite Havana SR1 XylT gene variant 1.

SEQ ID NO: 4: partial amino acid sequence of *Nicotiana tabacum* cv. Petite Havana SR1 XylT protein variant 1.

SEQ ID NO: 5: partial cDNA sequence of *Nicotiana tabacum* cv. Petite Havana SR1 XylT gene variant 2.

SEQ ID NO: 6: partial amino acid sequence of *Nicotiana tabacum* cv. Petite Havana SR1 XylT protein variant 2.

SEQ ID NO: 7: partial nucleotide sequence of *Nicotiana tabacum* cv. Petite Havana SR1 XylT gene variant 1.

SEQ ID NO: 8: partial amino acid sequence of *Nicotiana tabacum* cv. Petite Havana SR1 XylT protein variant 1.

SEQ ID NO: 9: partial nucleotide sequence of *Nicotiana tabacum* cv. Petite Havana SR1 XylT gene variant 2.

SEQ ID NO: 10: partial amino acid sequence of *Nicotiana tabacum* cv. Petite Havana SR1 XylT protein variant 2.

SEQ ID NO: 11: partial nucleotide sequence of *Nicotiana benthamiana* XylT gene variant 1.

SEQ ID NO: 12: partial amino acid sequence of *Nicotiana benthamiana* XylT protein variant 1.

SEQ ID NO: 13: partial nucleotide sequence of *Nicotiana benthamiana* XylT gene variant 2.

SEQ ID NO: 14: partial amino acid sequence of *Nicotiana benthamiana* XylT protein variant 2.

SEQ ID NO: 15: nucleotide sequence of the oligonucleotide XylF8 suitable to amplify a part of a *Nicotiana tabacum* cv. Petite Havana SR1 XylT gene or cDNA.

SEQ ID NO: 16: nucleotide sequence of the oligonucleotide XylR8 suitable to amplify a part of a *Nicotiana tabacum* cv. Petite Havana SR1 XylT gene or cDNA.

SEQ ID NO: 17: partial cDNA sequence of *Nicotiana tabacum* cv. Petite Havana SR1 XylT gene variant 1.

SEQ ID NO: 18: nucleotide sequence of T-DNA region of vector pTKW20.

SEQ ID NO: 19: nucleotide sequence of the oligonucleotide XylF9 suitable to amplify a part of a *Nicotiana benthamiana* XylT gene or cDNA.

SEQ ID NO: 20: nucleotide sequence of the oligonucleotide XylR9 suitable to amplify a part of a *Nicotiana benthamiana* XylT gene or cDNA.

SEQ ID NO: 21: partial sequence of *Nicotiana benthamiana* XylT gene variant 1.

SEQ ID NO: 22: nucleotide sequence of T-DNA region of vector pTKW29.

SEQ ID NO: 23: *Nicotiana tabacum* cv. Xanthi mRNA for putative beta-(1,2)-xylosyltransferase (accession number AJ627182)

SEQ ID NO: 24: putative beta-(1,2)-xylosyltransferase encoded by SEQ ID NO:23

SEQ ID NO: 25: *Nicotiana tabacum* cv. Xanthi xylt gene for putative beta-(1,2)-xylosyltransferase (accession number AJ627183)

SEQ ID NO: 26: putative beta-(1,2)-xylosyltransferase encoded by SEQ ID NO:25

Examples

Example 1: Design of degenerated primers for the isolation of XylT cDNA and gene sequences from *Nicotiana tabacum* cv. Petite Havana SR1 and *Nicotiana benthamiana*

Oligonucleotide sequences to be used as degenerated primers in a PCR amplification of XylT cDNA and genomic DNA from *Nicotiana tabacum* cv. Petite Havana SR1 and *Nicotiana benthamiana* were designed based on exon sequences of a genomic DNA sequence from *Nicotiana tabacum* cv. Xanthi encoding a putative XylT protein (accession number AJ627183). The forward primer (SEQ ID NO:1) was designed with CACC at its 5' end for cloning purposes. In this way the following degenerated primers were generated:

XylF4: 5'-CACCTTGTTTCTCTCTTCGCTCTCAACTCAATCACTC-3'
(SEQ ID NO: 1)

XylR4: 5'-TCGATCACAACTGGAGGATCCGCATAA -3'
(SEQ ID NO: 2)

Example 2: Isolation of XylT cDNA sequences from *Nicotiana tabacum* cv. Petite Havana SR1

The degenerated primers described in Example 1 were used to isolate XylT cDNA sequences from *Nicotiana tabacum* cv. Petite Havana SR1:

RNA was extracted from leaves of *Nicotiana tabacum* cv. Petite Havana SR1 using the RNeasy Plant Mini Kit (Qiagen) according to the manufacturer's protocol and used for cDNA synthesis using SuperScript™ First-strand synthesis System for RT-PCR (Invitrogen Life Technologies) according to the manufacturer's instructions.

Using the cDNA as template and primer pair XylF4 / XylR4, PCR amplification was performed under the following conditions: 15 sec at 94°C (denaturation) and 3 min at 68°C for 40 cycles (annealing and elongation).

A DNA fragment of about 1500 basepairs was amplified, cloned into a pENTR™/D-TOPO® vector (Invitrogen) and several clones were sequenced (comprising the sequences of SEQ ID NO: 3 - XylTc2Nt - and SEQ ID NO: 5 - XylTc7Nt).

An alignment between a mRNA sequence from *Nicotiana tabacum* cv. Xanthi encoding a putative XylT protein (accession number AJ627182; SEQ ID NO:23) and the XylT cDNA sequences isolated from *Nicotiana tabacum* cv. Petite Havana SR1 (SEQ ID NO: 3 and 5) is shown in Figure 1.

An alignment between the putative XylT protein encoded by the mRNA sequence from *Nicotiana tabacum* cv. Xanthi (accession number AJ627182; SEQ ID NO:24) and by the cDNA sequences isolated from *Nicotiana tabacum* cv. Petite Havana SR1 (SEQ ID NO: 4 and 6) is shown in Figure 2.

Example 3: Isolation of XylT gene sequences of *Nicotiana tabacum* cv. Petite Havana SR1 and of *Nicotiana benthamiana*

The degenerated primers described in Example 1 were used to isolate XylT gene sequences from *Nicotiana tabacum* cv. Petite Havana SR1 and from *Nicotiana benthamiana*:

DNA was extracted from leaves of *Nicotiana tabacum* cv. Petite Havana SR1 and of *Nicotiana benthamiana* based on the protocol described by Bernatzky and Tanksley (1986).

Using the genomic DNA as template and primer pair XylF4 / XylR4, PCR amplification was performed under the following conditions: 15 sec at 94°C (denaturation) and 4 min 30 sec at 68°C for 40 cycles (annealing and elongation).

Using the genomic DNA from *Nicotiana tabacum* cv. Petite Havana SR1 as template for the PCR amplification, a DNA fragment of about 3400 basepairs was amplified, cloned into a pENTR™/D-TOPO® vector (Invitrogen) and several clones were sequenced (comprising the sequences of SEQ ID NO: 7 – XylTg1Nt - and SEQ ID NO: 9 – XylTg3Nt).

The XylT genomic DNA sequences XylTg1Nt and XylTg3Nt comprise two putative intron sequences and three putative exon sequences. The location of the intron sequences are:

- For XylTg1Nt: from the nucleotide at position 679 to the nucleotide at position 1974 and from the nucleotide at position 2125 to the nucleotide at position 2722 of SEQ ID NO: 7, and
- For XylTg3Nt: from the nucleotide at position 658 to the nucleotide at position 1953 and from the nucleotide at position 2104 to the nucleotide at position 2701 of SEQ ID NO: 9.

Using the genomic DNA from *Nicotiana benthamiana* as template for the PCR amplification, a DNA fragment of between about 3300 and about 3600 basepairs was amplified, cloned into a pENTR™/D-TOPO® vector (Invitrogen) and several clones were sequenced (comprising the sequences of SEQ ID NO: 11 – XylTg14Nb - and SEQ ID NO:13 – XylTg19Nb).

The XylT genomic DNA sequences XylTg14Nb and XylTg19Nb comprise two putative intron sequences and three putative exon sequences. The location of the intron sequences is:

- XylTg14Nb from the nucleotide at position 658 to the nucleotide at position 1917 and from the nucleotide at position 2068 to the nucleotide at position 2612 of SEQ ID NO: 11, and
- XylTg19Nb from the nucleotide at position 649 to the nucleotide at position 2194 and from the nucleotide at position 2345 to the nucleotide at position 2888 of SEQ ID NO: 13.

An alignment between the genomic DNA sequence from *Nicotiana tabacum* cv. Xanthi encoding a putative XylT protein (accession number AJ627183; SEQ ID NO:25) and the XylT genomic DNA sequences isolated from *Nicotiana tabacum* cv. Petite Havana SR1 (SEQ ID NO: 7 and 9) and from *Nicotiana benthamiana* (SEQ ID NO: 11 and 13) is shown in Figure 3.

An alignment between the putative XylT protein encoded by the genomic DNA sequence from *Nicotiana tabacum* cv. Xanthi (accession number AJ627183; SEQ ID NO:26) and by the

genomic DNA sequences isolated from *Nicotiana tabacum* cv. Petite Havana SR1 (SEQ ID NO:8 and 10) and from *Nicotiana benthamiana* (SEQ ID NO: 12 and 14) is shown in Figure 4.

Example 4: Construction of a T-DNA vector containing a *Nicotiana* XylT silencing gene

DNA fragments amplified from *Nicotiana* XylT sequences described in Examples 2 and 3 were used to construct T-DNA vectors comprising a chimeric gene which upon transcription yields an RNA molecule comprising a sense and antisense DNA sequence from the amplified DNA fragment, and which could basepair to form a double stranded RNA molecule. Such chimeric genes can be used to reduce the expression of a XylT gene in *Nicotiana*, particularly in *Nicotiana tabacum* cv. Petite Havana SR1 and *Nicotiana benthamiana*.

4.1. Construction of a T-DNA vector comprising a XylT silencing gene with a DNA fragment amplified from a XylT sequence from *Nicotiana tabacum* cv. Petite Havana SR1

Oligonucleotide sequences to be used as non-degenerated primers in a PCR amplification of a XylT cDNA sequence from *Nicotiana tabacum* cv. Petite Havana SR1 were designed based on the cDNA sequence from *Nicotiana tabacum* cv. Petite Havana SR1 isolated in Example 2. The forward primer (SEQ ID NO:15) was designed with CACC at its 5' end for cloning purposes. In this way the following non-degenerated primers were generated:

XylF8: 5'-CACCTCTCGCCTTTGGGATATGAAACT -3' (SEQ ID NO: 15)
XylR8: 5'-ACAGCTTTGGTGCTGCAGAAACT -3' (SEQ ID NO: 16)

Using the vector comprising a DNA fragment amplified from a XylT cDNA sequence of *Nicotiana tabacum* cv. Petite Havana SR1 as described in Example 2 (SEQ ID NO:3 - XylTc2Nt) as template and primer pair XylF8 / XylR8, a PCR amplification was performed under the following conditions: 15 sec at 94°C (denaturation), 30 sec at 56°C (annealing) and 45 sec at 68°C (elongation) for 25 cycles.

A DNA fragment of about 470 bp (XylTi4Nt; SEQ ID NO: 17) was amplified and cloned into a pENTR™/D-TOPO® vector (Invitrogen) yielding plasmid pKW19. Plasmid pKW19 was

recombined with pHellsgate12 (Helliwell and Waterhouse, Methods (2003) 30: 289-295) using Gateway® LR Clonase™ II (Invitrogen) yielding plasmid pTKW20.

The T-DNA sequence of pTKW20 (SEQ ID NO: 18) thus comprises:

- A chimeric XylT silencing gene comprising:
  - a fragment including the promoter region of the Cauliflower Mosaic Virus 35S transcript (Odell *et al.*, 1985)
    (SEQ ID NO:18 from nucleotide 969 to nucleotide 2314)
  - a fragment including a part of the *Nicotiana tabacum* cv. Petite Havana SR1 XylT cDNA sequence cloned in sense orientation
    (SEQ ID NO:18 from nucleotide 2365 to nucleotide 2834)
  - a fragment containing the intron of the catase-1 gene from castor bean
    (SEQ ID NO:18 from nucleotide 2893 to nucleotide 3088)
  - a fragment containing the second intron of the pyruvate orthophosphate dikinase gene from *Flaveria trinervia* as described by Rosche and Westhoff (1995) in reverse orientation
    (SEQ ID NO:18 from nucleotide 3130 to nucleotide 3871).
  - a fragment including a part of the *Nicotiana tabacum* cv. Petite Havana SR1 XylT cDNA sequence cloned in antisense orientation
    (SEQ ID NO:18 from nucleotide 3957 to nucleotide 4426).
  - a fragment including the 3′ untranslated region of the octopine synthase gene of *Agrobacterium tumefaciens* as described by De Greve *et al.* (1982)
    (SEQ ID NO:18 from nucleotide 4479 to nucleotide 5244).
- A chimeric gene encoding a selectable marker comprising:
  - a fragment including the promoter region of the nopaline synthase gene of *Agrobacterium tumefaciens* T-DNA
    (SEQ ID NO:18 from nucleotide 5512 to nucleotide 5744).
  - a fragment including the *npt*II antibiotic resistance gene
    (SEQ ID NO:18 from nucleotide 5745 to nucleotide 6690).
  - A fragment including the 3′ untranslated region of the nopaline synthase gene of *A. tumefaciens* T-DNA.

(SEQ ID NO:18 from nucleotide 6691 to nucleotide 7396).

The T-DNA vector was introduced into *Agrobacterium tumefaciens* comprising a helper Ti-plasmid.

4.2. Construction of a T-DNA vector comprising a XylT silencing gene with a DNA fragment amplified from a XylT sequence from *Nicotiana benthamiana*

Oligonucleotide sequences to be used as non-degenerated primers in a PCR amplification of a XylT gene sequence from *Nicotiana benthamiana* were designed based on the gene sequence from *Nicotiana benthamiana* isolated in Example 3. The forward primer (SEQ ID NO:19) was designed with GGCCGGATCCTCG at its 5' end and the reverse primer (SEQ ID NO:20) was designed with GGCCATCGATGGTACC at its 5' end for cloning purposes. In this way the following non-degenerated primers were generated:

XylF9: 5'-GGCCGGATCCTCGAGACACAATTGGAGGAAACATGGAAAGC-3'
(SEQ ID NO: 19)

XylR9: 5'-GGCCATCGATGGTACCGGCCCAGCTCTTTATGGAATCAAA -3'
(SEQ ID NO: 20)

Using the vector comprising a DNA fragment amplified from a XylT gene sequence of *Nicotiana benthamiana* as described in Example 3 (SEQ ID NO:11 - XylTg14Nb) as template and primer pair XylF9 / XylR9, a PCR amplification was performed under the following conditions: 15 sec at 94°C (denaturation), 30 sec at 58°C (annealing) and 30 sec at 68°C (elongation) for 25 cycles.

A DNA fragment of about 430 bp (XylTiNb; SEQ ID NO: 21) was amplified and digested with XhoI and KpnI and with BamHI and ClaI, respectively. The XhoI / KpnI and the BamHI / ClaI digested fragments were cloned in pHANNIBAL (Helliwell and Waterhouse, 2003) digested with XhoI / KpnI and BamHI / ClaI yielding pKW28.

Plasmid pKW28 thus comprises a chimeric XylT silencing gene comprising:

- a fragment including the promoter region of the Cauliflower Mosaic Virus 35S transcript (Odell *et al.*, 1985)
  (SEQ ID NO:22 from nucleotide 3779 to nucleotide 2434)
- a fragment including a part of the *Nicotiana benthamiana* XylT gene sequence cloned in sense orientation
  (SEQ ID NO:22 from nucleotide 2427 to nucleotide 2023).
- a fragment containing the second intron of the pyruvate orthophosphate dikinase gene from *Flaveria trinervia* as described by Rosche and Westhoff (1995)
  (SEQ ID NO:22 from nucleotide 1991 to nucleotide 1250).
- a fragment including a part of the *Nicotiana benthamiana* XylT gene sequence cloned in antisense orientation
  (SEQ ID NO:22 from nucleotide 1211 to nucleotide 807).
- a fragment including the 3′ untranslated region of the octopine synthase gene of *Agrobacterium tumefaciens* as described by De Greve *et al.* (1982)
  (SEQ ID NO:22 from nucleotide 786 to nucleotide 76).

between restriction sites MscI and PstI.

Plasmid pKW28 is digested with MscI and PstI and the chimeric gene is introduced between the T-DNA borders of a T-DNA vector cut with PstI and SmaI together with a chimeric gene encoding a selectable marker comprising:

- a fragment including the promoter region of the nopaline synthase gene of *A. tumefaciens* T-DNA
  (SEQ ID NO:22 from nucleotide 3854 to nucleotide 4140).
- a fragment including the *bar* phosphinothricin resistance gene (De Block *et al.*, 1987)
  (SEQ ID NO:22 from nucleotide 4161 to nucleotide 4712).
- a fragment including the 3′ untranslated region of the nopaline synthase gene of *A. tumefaciens* T-DNA
  (SEQ ID NO:22 from nucleotide 4731 to nucleotide 4991).

to yield pTKW29 (sequence of the T-DNA of pTKW29 is represented in SEQ ID NO: 22).

The vector pTKW29 is derived from pGSC1700 (Cornelissen and Vandewiele, 1989). The vector backbone contains the following genetic elements:

- the plasmid core comprising the origin of replication from the plasmid pBR322 (Bolivar *et al.*, 1977) for replication in *Escherichia coli* (ORI ColE1) and a restriction fragment comprising the origin of replication from the *Pseudomonas* plasmid pVS1 (Itoh *et al.*, 1984) for replication in *Agrobacterium tumefaciens* (ORI pVS1).
- a selectable marker gene conferring resistance to streptomycin and spectinomycin (aadA) for propagation and selection of the plasmid in *Escherichia coli* and *Agrobacterium tumefaciens*.
- a DNA region consisting of a fragment of the neomycin phosphotransferase coding sequence of the *nptI* gene from transposon *Tn*903 (Oka *et al.*, 1981).

The T-DNA vector is introduced into *Agrobacterium tumefaciens* comprising a helper Ti-plasmid.

Example 5: Analysis of transgenic *Nicotiana* plants harboring a XylT silencing gene.

Nicotiana plants were transformed using the *Agrobacterium tumefaciens* strains described in Example 4:

5.1. Analysis of transgenic *Nicotiana tabacum* cv. Petite Havana SR1 plants harboring a XylT silencing gene

Nicotiana tabacum cv. Petite Havana SR1 plants were transformed using the *Agrobacterium tumefaciens* strain described in Example 4.1. according to the protocol as described in Zambryski *et al.* (1983). Fifty-two transgenic *Nicotiana tabacum* lines, comprising the chimeric genes as described in Example 4.1. were obtained.

Transgenic plant lines were analyzed on molecular level using Southern blot analysis. Similarly, the plant lines are analyzed for XylT RNA expression using Northern blot analysis.

An indication of XylT activity can be obtained by comparing the level of beta-1,2-xylose residues present on the glycans of proteins from the transgenic lines with that of untransformed plants. The level of beta-1,2-xylose residues on protein-bound N-glycans of plants can be measured e.g. by Western blot analysis using xylose-specific antibodies as described e.g. by Faye *et al.* (1993) or by mass spectrometry on glycans isolated from the plant's glycoproteins using Matrix-assisted Laser Desorption/Ionization Time-of-Flight mass spectronomy (MALDI-TOF-MS) as described e.g. by Kolarich and Altmann (2000) or using Liquid Chromatography Tandem mass spectronomy (LC/MS/MS) as described e.g. by Henriksson *et al.* (2003).

5.2. Analysis of transgenic *Nicotiana benthamiana* plants harboring a XylT silencing gene

Similarly, *Nicotiana benthamiana* plants were transformed using the *Agrobacterium tumefaciens* strain described in Example 4.2. and the expression of XylT and the level of beta-1,2-xylose residues present on the glycans of proteins was analyzed as described above.

Fifty four transgenic *Nicotiana benthamiana* lines comprising the chimeric genes described in Example 4.2. were obtained after leaf disk transformation with pTKW29.

To determine the level of beta-1,2-xylose residues present on the glycans of endogenous proteins of these plant lines, soluble leaf proteins of each individual were analyzed by Western blot using a beta-1,2-xylose-specific antibody.

Six samples showed very weak reaction with the antibody and six samples had no detectable reaction with the antibody. For the other samples, the level of reaction with the antibody ranged from weak to wild-type level.

To determine the number of insertions of the chimeric XylT silencing gene from pTKW29, genome DNA from the plant lines showing very weak or negative reactions to the beta-1,2-xylose-specific antibody was isolated, digested with EcoRI and analyzed by Southern blot using a probe spanning the 35S promoter region and a probe spanning the bar phosphinotricin resistance gene's coding region.

None of the twelve plant lines showed a single insertion. One plant line contained two insertions and was negative for xylose using Western blot analysis.

To test whether these two chimeric XylT silencing genes inserted independently and to obtain plants which are negative for xylose on Western blot and which contain a single chimeric XylT silencing gene, progeny resulting from self fertilization of the plant line containing two insertions were sown and twenty five plant lines were analyzed by Western blot analysis.

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WE CLAIM

1) A method to produce a *Nicotiana* plant cell or plant having a low level of beta-1,2-xylose residues on protein-bound N-glycans comprising the steps of

a) introducing a chimeric gene into plant cells of a *Nicotiana* species or cultivar to generate transgenic plant cells, said chimeric gene comprising the following operably linked DNA fragments:

i) a plant expressible promoter;

ii) a transcribable DNA region comprising

(1) a first sense DNA region comprising a nucleotide sequence of at least 19 out of 20 consecutive nucleotides selected from a nucleotide sequence encoding a *Nicotiana* XylT protein, or the complement thereof, said nucleotide sequence preferably obtainable from said *Nicotiana* species or cultivar, wherein said at least 19 out of 20 consecutive nucleotides encode at least one *Nicotiana* species- or cultivar-specific XylT amino acid, or selected from a nucleotide sequence of a *Nicotiana* XylT gene or a *Nicotiana* XylT cDNA, or the complement thereof, said nucleotide sequence preferably obtainable from said *Nicotiana* species or cultivar, wherein said at least 19 out of 20 consecutive nucleotides comprise at least one *Nicotiana* species-specific XylT nucleotide;

(2) a second antisense DNA region comprising a nucleotide sequence of at least 19 consecutive nucleotides which have at least 95% sequence identity to the complement of said first DNA region;

wherein an RNA molecule transcribed from said transcribable DNA region is capable of forming a double stranded RNA region at least between an RNA region transcribed from said first sense DNA region and an RNA region transcribed from said second antisense DNA region; and

iii) a DNA region comprising a transcription termination and polyadenylation signal functional in plants;

b) optionally, identifying a transgenic *Nicotiana* plant cell which has a lower level of beta-1,2-xylose residues on protein-bound N-glycans than an untransformed *Nicotiana* plant cell;

c) optionally, regenerating said transgenic *Nicotiana* plant cells to obtain transgenic *Nicotiana* plants; and

d) optionally, identifying a transgenic *Nicotiana* plant which has a lower level of beta-1,2-xylose residues on protein-bound N-glycans than an untransformed *Nicotiana* plant.

2) The method of claim 1, wherein said *Nicotiana* species-specific XylT amino acid is a *Nicotiana benthamiana*-specific XylT amino acid and said *Nicotiana* species is preferably *Nicotiana benthamiana*.

3) The method of claim 1 or 2, wherein said nucleotide sequence encoding a *Nicotiana* XylT protein comprises a nucleotide sequence encoding the amino acid sequence of SEQ ID No.: 12 or SEQ ID No.:14.

4) The method of any one of claims 1 to 3, wherein said *Nicotiana* species-specific XylT nucleotide is a *Nicotiana benthamiana*-specific XylT nucleotide and said *Nicotiana* species is preferably *Nicotiana benthamiana*.

5) The method of any one of claims 1 to 4, wherein said nucleotide sequence of said *Nicotiana* XylT gene comprises the nucleotide sequence of SEQ ID No.: 11, SEQ ID No.:13, or SEQ ID No. 21.

6) The method of claim 1, wherein said *Nicotiana* cultivar-specific XylT amino acid is a *Nicotiana tabacum* cv. Petite Havana SR1-specific XylT amino acid and said *Nicotiana* cultivar is preferably *Nicotiana tabacum* cv. Petite Havana SR1.

7) The method of claim 1 or 6, wherein said nucleotide sequence encoding said *Nicotiana* XylT protein comprises a nucleotide sequence encoding the amino acid sequence of SEQ ID No.: 4, SEQ ID No.:6, SEQ ID No.: 8 or SEQ ID No.:10.

8) The method of any one of claims 1, 6 or 7, wherein said *Nicotiana* cultivar-specific XylT nucleotide is a *Nicotiana tabacum* cv. Petite Havana SR1-specific XylT nucleotide and said *Nicotiana* cultivar is preferably *Nicotiana tabacum* cv. Petite Havana SR1.

9) The method of any one of claims 1 or 6 to 8, wherein said nucleotide sequence of said *Nicotiana* XylT gene or said *Nicotiana* XylT cDNA comprises the nucleotide sequence of SEQ ID No.: 3, SEQ ID No.: 5, SEQ ID No.: 8, SEQ ID No.:10, or SEQ ID No.: 17.

10) The method of any one of claims 1 to 9, wherein said first and said second DNA region comprise at least 50 consecutive nucleotides.

11) The method of any one of claims 1 to 9, wherein said first and said second DNA region comprise at least 200 consecutive nucleotides.

12) A method to produce a *Nicotiana* plant cell or plant having a low level of beta-1,2-xylose residues on protein-bound N-glycans comprising the steps of:

a) providing one or more double stranded RNA molecules to plant cells or plants of a *Nicotiana* species or cultivar, wherein the double stranded RNA molecules comprise two RNA strands, one RNA strand consisting essentially of an RNA nucleotide sequence of 19 out of 20 to 21 consecutive nucleotides selected from a nucleotide sequence encoding a *Nicotiana* XylT protein, or the complement thereof, said nucleotide sequence preferably obtainable from said *Nicotiana* species or cultivar, wherein said 19 out of 20 to 21 consecutive nucleotides encode at least one *Nicotiana* species- or cultivar-specific XylT amino acid, or selected from the nucleotide sequence of a *Nicotiana* XylT gene or a *Nicotiana* XylT cDNA, or the complement thereof, said nucleotide sequence preferably obtainable from said *Nicotiana* species or cultivar, wherein said 19 out of 20 to 21 consecutive nucleotides comprise at least one *Nicotiana* species- or cultivar-specific XylT nucleotide;

b) identifying a *Nicotiana* plant cell or plant comprising said double stranded RNA molecule or molecules which has a lower level of beta-1,2-xylose residues on protein-

bound N-glycans than a same *Nicotiana* plant cell or plant which does not comprise said double stranded RNA molecule or molecules.

13) The method of claim 12, wherein said double stranded RNA is provided to said plant cells or plants by integrating a chimeric gene into the genome of plant cells of said *Nicotiana* species or cultivar to generate transgenic plant cells and, optionally, regenerating said plant cells to obtain transgenic plants, said chimeric gene comprising the following operably linked DNA fragments:

a) a plant expressible promoter;

b) a DNA region comprising at least 19 out of 20 consecutive nucleotides selected from a nucleotide sequence encoding a *Nicotiana* XylT protein, or the complement thereof, said nucleotide sequence preferably obtainable from said *Nicotiana* species or cultivar, wherein said 19 out of 20 consecutive nucleotides encode at least one *Nicotiana* species- or cultivar-specific XylT amino acid, or selected from the nucleotide sequence of a *Nicotiana* XylT gene or a *Nicotiana* XylT cDNA, or the complement thereof, said nucleotide sequence preferably obtainable from said *Nicotiana* species or cultivar, wherein said 19 out of 20 consecutive nucleotides comprise at least one *Nicotiana* species-specific XylT nucleotide, in antisense orientation;

c) a DNA region comprising a transcription termination and polyadenylation signal functional in plants.

14) The method of claim 12, wherein said double stranded RNA is provided to said plant cells or plants by integrating a chimeric gene into the genome of said plant cells to generate transgenic plant cells and, optionally, regenerating said plant cells to obtain transgenic plants, said chimeric gene comprising the following operably linked DNA fragments:

a) a plant expressible promoter;

b) a DNA region comprising at least 19 out of 20 consecutive nucleotides selected from a nucleotide sequence encoding a *Nicotiana* XylT protein, or the complement thereof, said nucleotide sequence preferably obtainable from said *Nicotiana* species or cultivar, wherein said 19 out of 20 consecutive nucleotides encode at least one *Nicotiana* species- or cultivar-specific XylT amino acid it complements, or selected from the

nucleotide sequence of a *Nicotiana* XylT gene or a *Nicotiana* XylT cDNA, or the complement thereof, said nucleotide sequence preferably obtainable from said *Nicotiana* species or cultivar, wherein said 19 out of 20 consecutive nucleotides comprise at least one *Nicotiana* species-specific XylT nucleotide, in sense orientation;

c) a DNA region comprising a transcription termination and polyadenylation signal functional in plants.

15) The method of claim 12, wherein said double stranded RNA is provided to said plant cells or plants by integrating a chimeric gene into the genome of said plant cells to generate transgenic plant cells and, optionally, regenerating said plant cells to obtain transgenic plants, said chimeric gene comprising the following operably linked DNA fragments:

a) a plant expressible promoter;

b) a transcribable DNA region comprising:

i) a first DNA region comprising at least 19 out of 20 consecutive nucleotides selected from a nucleotide sequence encoding a *Nicotiana* XylT protein, or the complement thereof, said nucleotide sequence preferably obtainable from said *Nicotiana* species or cultivar, wherein said 19 out of 20 consecutive nucleotides encode at least one *Nicotiana* species- or cultivar-specific XylT amino acid, or selected from the nucleotide sequence of a *Nicotiana* XylT gene or a *Nicotiana* XylT cDNA, or the complement thereof, said nucleotide sequence preferably obtainable from said *Nicotiana* species or cultivar, wherein said 19 out of 20 consecutive nucleotides comprise at least one *Nicotiana* species-specific XylT nucleotide, in antisense orientation;

ii) a second DNA region comprising at least 19 out of 20 consecutive nucleotides selected from a nucleotide sequence encoding a *Nicotiana* XylT protein, or the complement thereof, said nucleotide sequence preferably obtainable from said *Nicotiana* species or cultivar, wherein said 19 out of 20 consecutive nucleotides encode at least one *Nicotiana* species- or cultivar-specific XylT amino acid, or selected from the nucleotide sequence of a *Nicotiana* XylT gene or a *Nicotiana* XylT cDNA, or the complement thereof, said nucleotide sequence preferably obtainable from said *Nicotiana* species or cultivar, wherein said 19 out of 20

consecutive nucleotides comprise at least one *Nicotiana* species-specific XylT nucleotide, in sense orientation,

whereby an RNA molecule produced by transcription of said transcribable DNA region is capable of forming a double stranded RNA region by base-pairing at least between an RNA region corresponding to said first DNA region and an RNA region corresponding to said second RNA region; and

c) a DNA region comprising a transcription termination and polyadenylation signal functional in plants.

16) The method of any one of claims 12 to 15, wherein said *Nicotiana* species-specific XylT amino acid is a *Nicotiana benthamiana*-specific XylT amino acid and said *Nicotiana* species is preferably *Nicotiana benthamiana.*

17) The method of any one of claims 12 to 16, wherein said nucleotide sequence encoding a *Nicotiana* XylT protein comprises a nucleotide sequence encoding the amino acid sequence of SEQ ID No.: 12 or SEQ ID No.:14.

18) The method of any one of claims 12 to 17, wherein said *Nicotiana* species-specific XylT nucleotide is a *Nicotiana benthamiana*-specific XylT nucleotide and said *Nicotiana* species is preferably *Nicotiana benthamiana.*

19) The method of any one of claims 12 to 18, wherein said nucleotide sequence of said *Nicotiana* XylT gene comprises the nucleotide sequence of SEQ ID No.: 11, SEQ ID No.:13, or SEQ ID No. 21.

20) The method of any one of claims 12 to 15, wherein said *Nicotiana* cultivar-specific XylT amino acid is a *Nicotiana tabacum* cv. Petite Havana SR1-specific XylT amino acid and said *Nicotiana* cultivar is preferably *Nicotiana tabacum* cv. Petite Havana SR1.

21) The method of any one of claims 12 to 15 or 20, wherein said nucleotide sequence encoding said *Nicotiana* XylT protein comprises a nucleotide sequence encoding the amino acid sequence of SEQ ID No.: 4, SEQ ID No.:6, SEQ ID No.: 8 or SEQ ID No.:10.

22) The method of any one of claims 12 to 15, 20 or 21, wherein said *Nicotiana* cultivar-specific XylT nucleotide is a *Nicotiana tabacum* cv. Petite Havana SR1-specific XylT nucleotide and said *Nicotiana* cultivar is preferably *Nicotiana tabacum* cv. Petite Havana SR1.

23) The method of any one of claims 12 to 15, or 20 to 22, wherein said nucleotide sequence of said *Nicotiana* XylT gene or said *Nicotiana* XylT cDNA comprises the nucleotide sequence of SEQ ID No.: 3, SEQ ID No.: 5, SEQ ID No.: 8, SEQ ID No.:10, or SEQ ID No.: 17.

24) A method to produce a *Nicotiana* plant cell or plant having a low level of beta-1,2-xylose residues on protein-bound N-glycans comprising the steps of:

a) identifying a fragment of a XylT protein encoding DNA sequence obtainable from a first *Nicotiana* species or cultivar, using a means selected from the following group:

i) a DNA fragment comprising a nucleotide sequence encoding the amino acid sequence of SEQ ID No.: 4, SEQ ID No.:6, SEQ ID No.: 8, SEQ ID No.:10, SEQ ID No.: 12, or SEQ ID No.:14, for use as a probe;

ii) a DNA fragment comprising the nucleotide sequence of any one of SEQ ID No.: 3, SEQ ID No.: 5, SEQ ID No.: 7, SEQ ID No.: 9, SEQ ID No.: 11, SEQ ID No.: 13, SEQ ID No.: 17, or SEQ ID No.: 21, for use as a probe;

iii) a DNA fragment or oligonucleotide comprising a nucleotide sequence consisting of between 20 to 1513 consecutive nucleotides selected from a nucleotide sequence encoding the amino acid sequence of SEQ ID No.: 4, or SEQ ID No.:6, for use as a probe;

iv) a DNA fragment or oligonucleotide comprising a nucleotide sequence consisting of between 20 to 3574 consecutive nucleotides selected from a nucleotide sequence

encoding the amino acid sequence of SEQ ID No.: 8, SEQ ID No.:10, SEQ ID No.: 12, or SEQ ID No.:14 for use as a probe

v) a DNA fragment or oligonucleotide comprising a nucleotide sequence consisting of between 20 to 3574 consecutive nucleotides selected from a nucleotide sequence of any one of SEQ ID No.: 3, SEQ ID No.: 5, SEQ ID No.: 7, SEQ ID No.: 9, SEQ ID No.: 11, SEQ ID No.: 13, SEQ ID No.: 17, or SEQ ID No.: 21 for use as a probe;

vi) an oligonucleotide sequence having a nucleotide sequence comprising between 20 to 200 consecutive nucleotides selected from a nucleotide sequence encoding the amino acid sequence of SEQ ID No.: 4, or SEQ ID No.:6, for use as a primer in a PCR reaction;

vii) an oligonucleotide sequence having a nucleotide sequence comprising between 20 to 200 consecutive nucleotides selected from a nucleotide sequence encoding the amino acid sequence of SEQ ID No.: 8, SEQ ID No.:10, SEQ ID No.: 12, or SEQ ID No.:14 , for use as a primer in a PCR reaction;

viii) an oligonucleotide sequence having a nucleotide sequence comprising between 20 to 200 consecutive nucleotides selected from the nucleotide sequence of any one of SEQ ID No.: 3, SEQ ID No.: 5, SEQ ID No.: 7, SEQ ID No.: 9, SEQ ID No.: 11, SEQ ID No.: 13, SEQ ID No.: 17, or SEQ ID No.: 21, for use as a primer in a PCR reaction; or

ix) an oligonucleotide having the nucleotide sequence of any one of SEQ ID No.: 1, SEQ ID No.: 2, SEQ ID No.: 15 or SEQ ID No.: 16, SEQ ID No.:19 or SEQ ID No.20 for use as a primer in a PCR reaction;

b) providing one or more double stranded RNA molecules to plant cells or plants of said first or a second *Nicotiana* species or cultivar, wherein said double stranded RNA molecules comprise two RNA strands, one RNA strand consisting essentially of an RNA nucleotide sequence of 20 to 21 consecutive nucleotides selected from a nucleotide sequence of said XylT protein encoding DNA fragment, or the complement thereof, wherein said 20 to 21 consecutive nucleotides encode at least one *Nicotiana* species- or cultivar-specific XylT amino acid, respectively, or wherein said 20 to 21 consecutive nucleotides comprise at least one *Nicotiana* species- or cultivar-specific XylT nucleotide, respectively; and

c) identifying a *Nicotiana* plant cell or plant comprising said double stranded RNA molecule or molecules which has a lower level of beta-1,2-xylose residues on protein-bound N-glycans than a same *Nicotiana* plant cell or plant, which does not comprise said double stranded RNA molecule or molecules.

25) The method of claim 24, wherein provision of said double stranded RNA molecule or molecules is achieved by providing to said plant cells or plants a double stranded RNA molecule or molecules comprising a first nucleotide sequence of at least 19 out of 20 consecutive nucleotides selected from the nucleotide sequence of said XylT protein encoding DNA fragment, or the complement thereof, wherein said at least 19 out of 20 consecutive nucleotides encode at least one *Nicotiana* species- or cultivar-specific XylT amino acid, or wherein said at least 19 out of 20 consecutive nucleotides comprise at least one *Nicotiana* species- or cultivar-specific XylT nucleotide, and a second nucleotide sequence which is the complement of said first nucleotide sequence.

26) The method of claim 24, wherein said double stranded RNA molecules are provided to said plant cells or plants by integrating a chimeric DNA into the genome of said plant cells to generate transgenic plant cells and, optionally, regenerating said plant cells to obtain transgenic plants, said chimeric DNA comprising the following operably linked DNA fragments:

a) a plant expressible promoter;

b) a transcribable DNA region comprising

i) a first sense DNA region comprising a nucleotide sequence of at least 19 out of 20 consecutive nucleotides selected from the nucleotide sequence of said XylT protein encoding DNA fragment, or the complement thereof, wherein said at least 19 out of 20 consecutive nucleotides encode at least one *Nicotiana* species- or cultivar-specific XylT amino acid, respectively, or wherein said at least 19 out of 20 consecutive nucleotides comprise at least one *Nicotiana* species- or cultivar-specific XylT nucleotide, respectively;

ii) a second antisense DNA region comprising a nucleotide sequence of at least 19 consecutive nucleotides which have at least 95% sequence identity to the complement of said first DNA region;

wherein an RNA molecule transcribed from said transcribable region is capable of forming a double stranded RNA region at least between an RNA region transcribed from said first sense DNA region and an RNA region transcribed from said second antisense DNA region; and

c) a DNA region comprising a transcription termination and polyadenylation signal functional in plants.

27) The method according to any one of claims 1 to 26 further comprising the step of crossing said *Nicotiana* plant having a low level of beta-1,2-xylose residues on protein-bound N-glycans to another *Nicotiana* plant to obtain *Nicotiana* progeny plants having a low level of beta-1,2-xylose residues on protein-bound N-glycans.

28) A method to identify a *Nicotiana* XylT DNA fragment, comprising the steps of

a) providing genomic DNA or cDNA obtainable from a *Nicotiana* species or cultivar;

b) selecting a means from the following group:

i) a DNA fragment comprising a nucleotide sequence encoding the amino acid sequence of SEQ ID No.: 4, SEQ ID No.:6, SEQ ID No.: 8, SEQ ID No.:10, SEQ ID No.: 12, or SEQ ID No.:14, for use as a probe;

ii) a DNA fragment comprising the nucleotide sequence of any one of SEQ ID No.: 3, SEQ ID No.: 5, SEQ ID No.: 7, SEQ ID No.: 9, SEQ ID No.: 11, SEQ ID No.: 13, SEQ ID No.: 17, or SEQ ID No.: 21, for use as a probe;

iii) a DNA fragment or oligonucleotide comprising a nucleotide sequence consisting of between 20 to 1513 consecutive nucleotides selected from a nucleotide sequence encoding the amino acid sequence of SEQ ID No.: 4, or SEQ ID No.:6, for use as a probe;

iv) a DNA fragment or oligonucleotide comprising a nucleotide sequence consisting of between 20 to 3574 consecutive nucleotides selected from a nucleotide sequence

encoding the amino acid sequence of SEQ ID No.: 8, SEQ ID No.:10, SEQ ID No.: 12, or SEQ ID No.:14 for use as a probe

v) a DNA fragment or oligonucleotide comprising a nucleotide sequence consisting of between 20 to 3574 consecutive nucleotides selected from a nucleotide sequence of any one of SEQ ID No.: 3, SEQ ID No.: 5, SEQ ID No.: 7, SEQ ID No.: 9, SEQ ID No.: 11, SEQ ID No.: 13, SEQ ID No.: 17, or SEQ ID No.: 21 for use as a probe;

vi) an oligonucleotide sequence having a nucleotide sequence comprising between 20 to 200 consecutive nucleotides selected from a nucleotide sequence encoding the amino acid sequence of SEQ ID No.: 4, or SEQ ID No.:6, for use as a primer in a PCR reaction;

vii) an oligonucleotide sequence having a nucleotide sequence comprising between 20 to 200 consecutive nucleotides selected from a nucleotide sequence encoding the amino acid sequence of SEQ ID No.: 8, SEQ ID No.:10, SEQ ID No.: 12, or SEQ ID No.:14 , for use as a primer in a PCR reaction;

viii) an oligonucleotide sequence having a nucleotide sequence comprising between 20 to 200 consecutive nucleotides selected from the nucleotide sequence of any one of SEQ ID No.: 3, SEQ ID No.: 5, SEQ ID No.: 7, SEQ ID No.: 9, SEQ ID No.: 11, SEQ ID No.: 13, SEQ ID No.: 17, or SEQ ID No.: 21, for use as a primer in a PCR reaction; or

ix) an oligonucleotide having the nucleotide sequence of any one of SEQ ID No.: 1, SEQ ID No.: 2, SEQ ID No.: 15 or SEQ ID No.: 16, SEQ ID No.:19 or SEQ ID No.20 for use as a primer in a PCR reaction;

c) identifying a XylT DNA fragment from said *Nicotiana* species or cultivar by performing a PCR using said genomic DNA or said cDNA and said primers, or by performing hybridization using said genomic DNA or said cDNA and said probes.

29) A method to isolate a *Nicotiana* XylT DNA fragment, comprising the steps of

a) identifying said *Nicotiana* XylT DNA fragment according to the method of claim 28; and

b) isolating said *Nicotiana* XylT DNA fragment.

30) A method to identify a *Nicotiana* XylT allele correlated with a low level of beta-1,2-xylose residues on protein-bound N-glycans comprising the steps of :

(a) providing a population, optionally a mutagenized population, of different plant lines of a *Nicotiana* species or cultivar;

(b) identifying in each plant line of said population a *Nicotiana* XylT allele according to the method of claim 28;

(c) analyzing the level of beta-1,2-xylose residues on protein-bound N-glycans of each plant line of said population and identifying those plant lines having a lower level of beta-1,2-xylose residues on protein-bound N-glycans than other plant lines;

(d) correlating the low level of beta-1,2-xylose residues on protein-bound N-glycans in a plant line to the presence of a specific *Nicotiana* XylT allele.

31) A method to obtain a *Nicotiana* plant cell or plant with a low level of beta-1,2-xylose residues on protein-bound N-glycans, comprising the steps of

a) identifying a *Nicotiana* XylT allele correlated with a low level of beta-1,2-xylose residues on protein-bound N-glycans according to the method of claim 30;

b) introducing said *Nicotiana* XylT allele into a *Nicotiana* plant line of choice.

32) An isolated DNA fragment encoding a protein comprising the amino acid sequence of SEQ ID No.: 12, or SEQ ID No.:14, or any part thereof encoding at least one *Nicotiana benthamiana*-specific XylT amino acid.

33) The isolated DNA fragment of claim 32, comprising the nucleotide sequence of SEQ ID No.: 11, SEQ ID No.: 13, or SEQ ID No.: 21, or any part thereof comprising at least one *Nicotiana benthamiana*-specific XylT nucleotide.

34) An isolated DNA fragment encoding a protein comprising the amino acid sequence of SEQ ID No.: 4 or SEQ ID No.:6, SEQ ID No.: 8, SEQ ID No.:10, or any part thereof encoding at least one *Nicotiana tabacum* cv. Petite Havana SR1-specfic XylT amino acid.

35) The isolated DNA fragment of claim 34, comprising the nucleotide sequence of SEQ ID No.: 3 or SEQ ID No.:5, SEQ ID No.: 7, SEQ ID No.:9, or SEQ ID No.: 17, or any part thereof comprising at least one *Nicotiana tabacum* cv. Petite Havana SR1-specfic XylT nucleotide.

36) An isolated DNA fragment obtainable by the method of claim 28, encoding at least one *Nicotiana* species- or *Nicotiana* cultivar-specific XylT amino acid.

37) The isolated DNA fragment of claim 36, comprising at least one *Nicotiana* species- or *Nicotiana* cultivar-specific XylT nucleotide.

38) A chimeric gene comprising the following operably linked DNA fragments:

a) a plant expressible promoter;

b) a transcribable DNA region comprising

i) a first DNA region comprising at least 19 out of 20 consecutive nucleotides selected from a nucleotide sequence encoding a *Nicotiana* XylT protein, or the complement thereof, wherein said 19 out of 20 consecutive nucleotides encode at least one *Nicotiana* species- or cultivar-specific XylT amino acid, or selected from the nucleotide sequence of a *Nicotiana* XylT gene or a *Nicotiana* XylT cDNA, or the complement thereof, wherein said 19 out of 20 consecutive nucleotides comprise at least one *Nicotiana* species-specific XylT nucleotide, in antisense orientation;

ii) a second DNA region comprising at least 19 out of 20 consecutive nucleotides selected from a nucleotide sequence encoding a *Nicotiana* XylT protein, or the complement thereof, wherein said 19 out of 20 consecutive nucleotides encode at least one *Nicotiana* species- or cultivar-specific XylT amino acid, or selected from the nucleotide sequence of a *Nicotiana* XylT gene or a *Nicotiana* XylT cDNA, or the complement thereof, wherein said 19 out of 20 consecutive nucleotides comprise at least one *Nicotiana* species-specific XylT nucleotide, in sense orientation,

whereby an RNA molecule produced by transcription of said transcribable DNA region is capable of forming a double stranded RNA region by base-pairing at least between

an RNA region corresponding to said first DNA region and an RNA region corresponding to said second RNA region; and

c) a DNA region comprising a transcription termination and polyadenylation signal functional in plants.

39) A chimeric gene comprising the following operably linked DNA fragments

a) a plant expressible promoter;

b) a DNA region comprising at least 19 out of 20 consecutive nucleotides selected from a nucleotide sequence encoding a *Nicotiana* XylT protein, or the complement thereof, wherein said 19 out of 20 consecutive nucleotides encode at least one *Nicotiana* species- or cultivar-specific XylT amino acid, or selected from the nucleotide sequence of a *Nicotiana* XylT gene or a *Nicotiana* XylT cDNA, or the complement thereof, wherein said 19 out of 20 consecutive nucleotides comprise at least one *Nicotiana* species-specific XylT nucleotide, in sense orientation; and

c) a DNA region comprising a transcription termination and polyadenylation signal functional in plants.

40) A chimeric gene comprising the following operably linked DNA fragments

a) a plant expressible promoter;

b) a DNA region comprising at least 19 out of 20 consecutive nucleotides selected from a nucleotide sequence encoding a *Nicotiana* XylT protein, or the complement thereof, wherein said 19 out of 20 consecutive nucleotides encode at least one *Nicotiana* species- or cultivar-specific XylT amino acid, or selected from the nucleotide sequence of a *Nicotiana* XylT gene or a *Nicotiana* XylT cDNA, or the complement thereof, wherein said 19 out of 20 consecutive nucleotides comprise at least one *Nicotiana* species-specific XylT nucleotide, in antisense orientation; and

c) a DNA region comprising a transcription termination and polyadenylation signal functional in plants.

41) The chimeric gene according to any one of claims 38 to 40, wherein said *Nicotiana* species-specific XylT amino acid is a *Nicotiana benthamiana*-specific XylT amino acid.

42) The chimeric gene according to any one of claims 38 to 41, wherein said nucleotide sequence encoding a *Nicotiana* XylT protein comprises a nucleotide sequence encoding the amino acid sequence of SEQ ID No.: 12 or SEQ ID No.:14.

43) The chimeric gene according to any one of claims 38 to 42, wherein said *Nicotiana* species-specific XylT nucleotide is a *Nicotiana benthamiana*-specific XylT nucleotide.

44) The chimeric gene according to any one of claims 38 to 43, wherein said nucleotide sequence of said *Nicotiana* XylT gene comprises the nucleotide sequence of SEQ ID No.: 11, SEQ ID No.:13, or SEQ ID No. 21.

45) The chimeric gene according to any one of claims 38 to 40, wherein said *Nicotiana* cultivar-specific XylT amino acid is a *Nicotiana tabacum* cv. Petite Havana SR1-specific XylT amino acid.

46) The chimeric gene according to any one of claims 38 to 40 or 45, wherein said nucleotide sequence encoding said *Nicotiana* XylT protein comprises a nucleotide sequence encoding the amino acid sequence of SEQ ID No.: 4, SEQ ID No.:6, SEQ ID No.: 8 or SEQ ID No.:10.

47) The chimeric gene according to any one of claims 38 to 40, 45 or 46, wherein said *Nicotiana* cultivar-specific XylT nucleotide is a *Nicotiana tabacum* cv. Petite Havana SR1-specific XylT nucleotide.

48) The chimeric gene according to any one of claims 38 to 40, or 45 to 47, wherein said nucleotide sequence of said *Nicotiana* XylT gene or said *Nicotiana* XylT cDNA comprises the nucleotide sequence of SEQ ID No.: 3, SEQ ID No.: 5, SEQ ID No.: 8, SEQ ID No.:10, or SEQ ID No.: 17.

49) A *Nicotiana* plant cell comprising the chimeric gene of any one of claims 38 to 48.

50) A *Nicotiana* plant consisting essentially of the *Nicotiana* plant cells of claim 49.

51) A *Nicotiana* plant cell or plant obtained by the method of claim 31.

52) A seed of a *Nicotiana* plant according to claim 50 or claim 51.

53) Use of a nucleotide sequence encoding a protein comprising the amino acid sequence of SEQ ID No.: 4, SEQ ID No.:6, SEQ ID No.: 8, SEQ ID No.:10, SEQ ID No.: 12, or SEQ ID No.:14, or any part thereof comprising at least 19 out of 20 consecutive nucleotides encoding at least one *Nicotiana* species- or cultivar-specific XylT amino acid, to decrease the level of beta-1,2-xylose residues on protein-bound N-glycans in a *Nicotiana* plant.

54) Use of a nucleotide sequence comprising the nucleotide sequence of SEQ ID No.: 3, SEQ ID No.:5, SEQ ID No.: 7, SEQ ID No.:9, SEQ ID No.: 11, SEQ ID No.: 13, SEQ ID No.: 17 or SEQ ID No.: 21, or any part thereof comprising at least 19 out of 20 consecutive nucleotides comprising at least one *Nicotiana* species- or cultivar-specific XylT nucleotide, to decrease the level of beta-1,2-xylose residues on protein-bound N-glycans in a *Nicotiana* plant.

55) Use of a nucleotide sequence encoding a protein comprising the amino acid sequence of SEQ ID No.: 4, SEQ ID No.:6, SEQ ID No.: 8, SEQ ID No.:10, SEQ ID No.: 12, or SEQ ID No.:14, or any part thereof encoding at least one *Nicotiana* species- or cultivar-specific XylT amino acid, to identify a XylT gene or XylT cDNA in a *Nicotiana* species or cultivar.

56) Use of a nucleotide sequence comprising the nucleotide sequence of SEQ ID No.: 3, SEQ ID No.:5, SEQ ID No.: 7, SEQ ID No.:9, SEQ ID No.: 11, SEQ ID No.: 13, SEQ ID No.: 17 or SEQ ID No.: 21, or any part thereof comprising at least one *Nicotiana* species- or cultivar-specific XylT nucleotide, to identify a XylT gene or XylT cDNA in a *Nicotiana* species or cultivar.

57) Use of a nucleotide sequence encoding a protein comprising the amino acid sequence of SEQ ID No.: 4, SEQ ID No.:6, SEQ ID No.: 8, SEQ ID No.:10, SEQ ID No.: 12, or SEQ ID No.:14, or any part thereof encoding at least one *Nicotiana* species- or cultivar-specific XylT amino acid, to identify an allele of a *XylT* gene correlated with a low level of beta-1,2-xylose residues on protein-bound N-glycans in a *Nicotiana* species or cultivar.

58) Use of a nucleotide sequence comprising the nucleotide sequence of SEQ ID No.: 3, SEQ ID No.:5, SEQ ID No.: 7, SEQ ID No.:9, SEQ ID No.: 11, SEQ ID No.: 13, SEQ ID No.: 17 or SEQ ID No.: 21, or any part thereof comprising at least one *Nicotiana* species- or cultivar-specific XylT nucleotide, to identify an allele of a *XylT* gene correlated with a low level of beta-1,2-xylose residues on protein-bound N-glycans in a *Nicotiana* species or cultivar.

59) Use of a nucleotide sequence encoding a protein comprising the amino acid sequence of SEQ ID No.: 4, SEQ ID No.:6, SEQ ID No.: 8, SEQ ID No.:10, SEQ ID No.: 12, or SEQ ID No.:14, or any part thereof encoding at least one *Nicotiana* species- or cultivar-specific XylT amino acid, to introduce an allele of a *XylT* gene correlated with a low level of beta-1,2-xylose residues on protein-bound N-glycans in a *Nicotiana* species or cultivar.

60) Use of a nucleotide sequence comprising the nucleotide sequence of SEQ ID No.: 3, SEQ ID No.:5, SEQ ID No.: 7, SEQ ID No.:9, SEQ ID No.: 11, SEQ ID No.: 13, SEQ ID No.: 17 or SEQ ID No.: 21, or any part thereof comprising at least one *Nicotiana* species- or cultivar-specific XylT nucleotide, to introduce an allele of a *XylT* gene correlated with a low level of beta-1,2-xylose residues on protein-bound N-glycans in a *Nicotiana* species or cultivar.

Figure 1

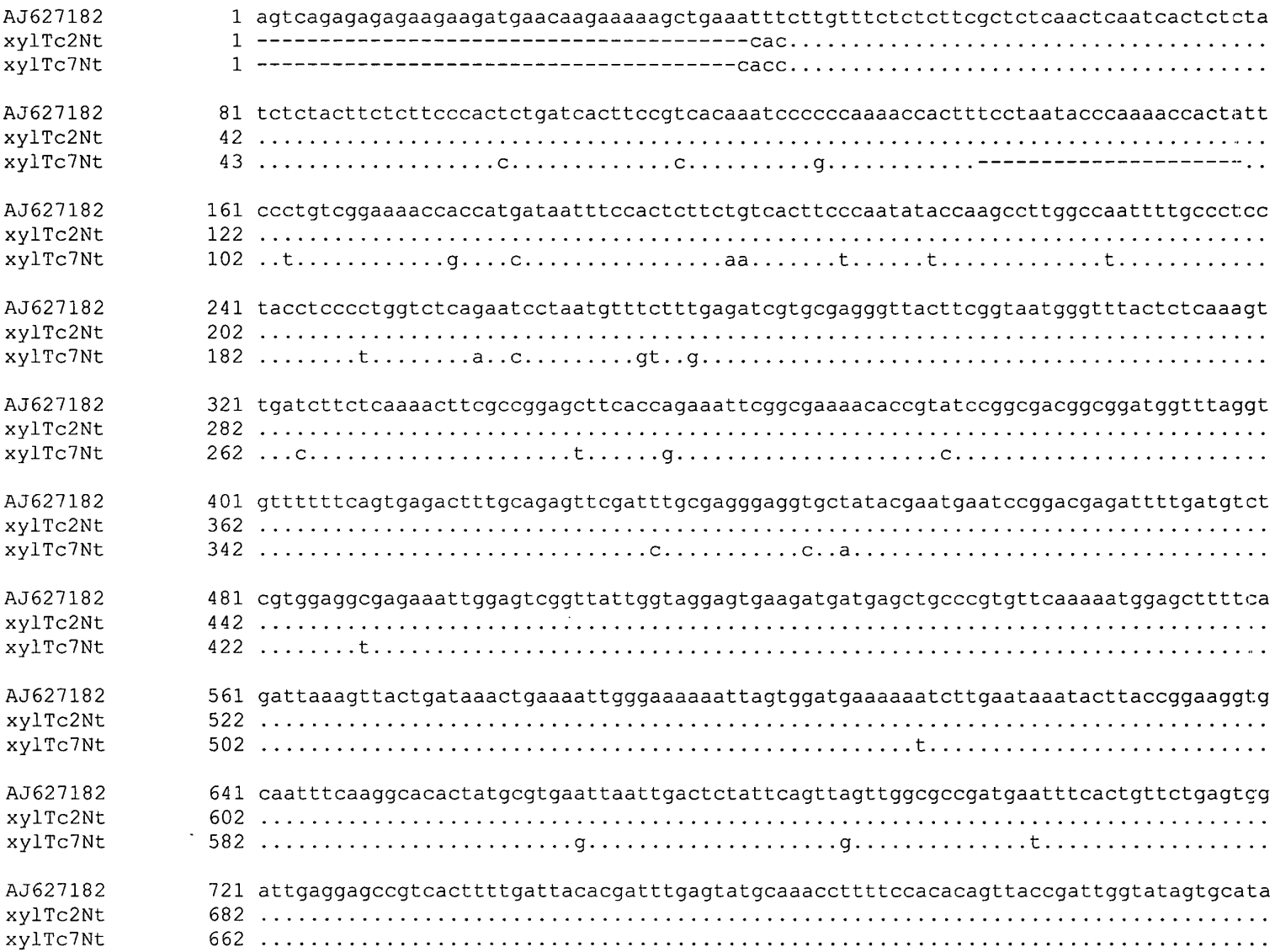
AJ627182 1 agtcagagagagaagaagatgaacaagaaaaagctgaaatttcttgtttctctcttcgctctcaactcaatcactctcta
xylTc2Nt 1 ----------cac.....
xylTc7Nt 1 ----------cacc.....
AJ627182 81 tctctacttctcttcccactctgatcacttccgtcacaaatccccccaaaaccactttcctaatacccaaaaccactatt
xylTc2Nt 42 .....
xylTc7Nt 43 .....c.....c.....g.....----------..
AJ627182 161 ccctgtcggaaaaccaccatgataatttccactcttctgtcacttcccaatataccaagccttggccaattttgccctcc
xylTc2Nt 122 .....
xylTc7Nt 102 ..t.....g....c.....aa.....t.....t.....t.....
AJ627182 241 tacctcccctggtctcagaatcctaatgtttctttgagatcgtgcgagggttacttcggtaatgggtttactctcaaagt
xylTc2Nt 202 .....
xylTc7Nt 182 .....t.....a..c.....gt..g.....
AJ627182 321 tgatcttctcaaaacttcgccggagcttcaccagaaattcggcgaaaacaccgtatccggcgacggcggatggtttaggt
xylTc2Nt 282 .....
xylTc7Nt 262 ...c.....t.....g.....c.....
AJ627182 401 gtttttcagtgagactttgcagagttcgatttgcgagggaggtgctatacgaatgaatccggacgagattttgatgtct
xylTc2Nt 362 .....
xylTc7Nt 342 .....c.....c..a.....
AJ627182 481 cgtggaggcgagaaattggagtcggttattggtaggagtgaagatgatgagctgcccgtgttcaaaaatggagcttttca
xylTc2Nt 442 .....
xylTc7Nt 422 .....t.....
AJ627182 561 gattaaagttactgataaactgaaaattgggaaaaaattagtggatgaaaaaatcttgaataaatacttaccggaaggtg
xylTc2Nt 522 .....
xylTc7Nt 502 .....t.....
AJ627182 641 caatttcaaggcacactatgcgtgaattaattgactctattcagttagttggcgccgatgaatttcactgttctgagtcg
xylTc2Nt 602 .....
xylTc7Nt 582 .....g.....g.....t.....
AJ627182 721 attgaggagccgtcacttttgattacacgatttgagtatgcaaacctttccacacagttaccgattggtatagtgcata
xylTc2Nt 682 .....
xylTc7Nt 662 .....

Figure 1 (continued)

```
AJ627182      801 cgtggcatccagggttactggcttgcccagtcggccacatttggtttttgtagatggccattgtgagacacaattggagg
xylTc2Nt      762 ...............................................................................
xylTc7Nt      742 ...............................................................................

AJ627182      881 aaacatggaaagcactcttttcaagcctcacttatgctaagaactttagtggcccagtttgtttccgtcacgccgttctc
xylTc2Nt      842 ...............................................................................
xylTc7Nt      822 ..............t..............................................................t....cc...

AJ627182      961 tcgcctttgggatatgaaactgccctgtttaagggactgacagaaactatagattgtaatggagcttctgcccatgattt
xylTc2Nt      922 ...............................................................................
xylTc7Nt      902 ....................................t..........................................

AJ627182     1041 gtggcaaaatcctgatgataagagaactgcacggttgtctgagtttggggagatgatcagggcagcctttggatttcctg
xylTc2Nt     1002 ....................................c..........................................
xylTc7Nt      982 ....................a.............c................t.....................

AJ627182     1121 tggatagacagaacatcccaaggacagtcacaggccctaatgtcctctttgttagacgtgaggattatttagctcaccca
xylTc2Nt     1082 ...............................................................................
xylTc7Nt     1062 ...............................................................................

AJ627182     1201 cgtcatggtggaaaggtacagtctaggcttagcaatgaagagcaagtatttgattccataaagagctgggccttgaacca
xylTc2Nt     1162 ...............................................................................
xylTc7Nt     1142 ...............................................................................

AJ627182     1281 ctcggagtgcaaattaaatgtaattaacggattgtttgcccacatgtccatgaaagagcaagttcgagcaatccaagatg
xylTc2Nt     1242 ...............................................................................
xylTc7Nt     1222 ...............................................................................

AJ627182     1361 cttctgtcatagttggtgctcatggagcaggtctaactcacatagtttctgcagcaccaaaagctgtaatactagaaatt
xylTc2Nt     1322 .a........t................................................................
xylTc7Nt     1302 ...............................................................................

AJ627182     1441 ataagcagcgaatataggcgccccattttgctctgattgcacaatggaaaggattggagtaccatcccatatatttgga
xylTc2Nt     1402 ..................a............................................................
xylTc7Nt     1382 ...............................................................................

AJ627182     1521 ggggtcttatgcggatcctccagttgtgatcgacaggctcagcagcattttgaggagtcttgggtgctaagtccgctcga
xylTc2Nt     1482 ........................------------------------------------------------
xylTc7Nt     1462 ..............................a------------------------------------------
```

Figure 1 (continued)

```
AJ627182      1601 cagtttgaatagttcggcttttctctaaaagacggggaaggatagaggaattcggggttctggaacttggagcctgggaa
xylTc2Nt           ----------------------------------------------------------------------------
xylTc7Nt           ----------------------------------------------------------------------------

AJ627182      1681 ttttgataaatatgtttcacacgcagttctgtagtcaatggttgcaatctaggtcctcaatctggtgttgataagcttgg
xylTc2Nt           ----------------------------------------------------------------------------
xylTc7Nt           ----------------------------------------------------------------------------

AJ627182      1761 caatttccagcagctactaatttattagcccgctctgactcggttatggactaccagagcaatcatatcaaatggaagca
xylTc2Nt           ----------------------------------------------------------------------------
xylTc7Nt           ----------------------------------------------------------------------------

AJ627182      1841 tggaatcctgattgtggaatggtgagctcattgaagagcatattctttatggtgttgaagattacaattcacaattaaca
xylTc2Nt           ----------------------------------------------------------------------------
xylTc7Nt           ----------------------------------------------------------------------------

AJ627182      1921 cgtgtatgtgaaagattaggttggtacacttacttacaattcattgtcaattgtttttcattattctcattaatgatcat
xylTc2Nt           ----------------------------------------------------------------------------
xylTc7Nt           ----------------------------------------------------------------------------

AJ627182      2001 aggataagaacatgagaaaaccatccatgttctgtgttgttttcccatcaatccggccaccctcttccctccttatgtag
xylTc2Nt           ----------------------------------------------------------------------------
xylTc7Nt           ----------------------------------------------------------------------------

AJ627182      2081 agatgatttcaacagagtttgttttgtagttgtaacacttgcactcccagttacagttttgcattcgacacattcatccc
xylTc2Nt      1509 -.....----------------------------------------------------------------------
xylTc7Nt           ----------------------------------------------------------------------------

AJ627182      2161 atcagatgtcaagtttaaaggcataagacatttgacatattgaagaagcagattaacacgaacgtcagtatgatgcttca
xylTc2Nt           ----------------------------------------------------------------------------
xylTc7Nt           ----------------------------------------------------------------------------

AJ627182      2241 gtgaagatatggttgtaacttgtaaccaaacaaaagaaatgagactttgacaaaaaaaaaaaaaaaaaaaaaaaaaa
xylTc2Nt           ----------------------------------------------------------------------------
xylTc7Nt           ----------------------------------------------------------------------------
```

Figure 2

```
AJ627182          1 mnkkklkflvslfalnsitlylyfsshsdhfrhkspqnhfpntqnhyslsenhhdnfhssvtsqytkpwpilpsylpwsq
xylTc2Nt          1 ---------.....................................................................
xylTc7Nt          1 --------.................p.......r....-------.....r.h.....i....s.............

AJ627182         81 npnvslrscegyfgngftlkvdllktspelhqkfgentvsgdggwfrcffsetlqssiceggairmnpdeilmsrggekl
xylTc2Nt         72 .....................................................................................
xylTc7Nt         66 ....vw.....................f.r.....................................................

AJ627182        161 esvigrseddelpvfkngafqikvtdklkigkklvdekilnkylpegaisrhtmrelidsiqlvgadefhcsewieepsl
xylTc2Nt        152 .....................................................................................
xylTc7Nt        146 ....................................f.............................d.....................

AJ627182        241 litrfeyanlfhtvtdwysayvasrvtglpsrphlvfvdghcetqleetwkalfssltyaknfsgpvcfrhavlsplgye
xylTc2Nt        232 .....................................................................................
xylTc7Nt        226 ....................................................................a........

AJ627182        321 talfkgltetidcngasahdlwqnpddkrtarlsefgemiraafgfpvdrqniprtvtgpnvlfvrredylahprhggkv
xylTc2Nt        312 .....................................................................................
xylTc7Nt        306 .......s.................k.....................................................

AJ627182        401 qsrlsneeqvfdsikswalnhsecklnvinglfahmsmkeqvraiqdasvivgahgaglthivsaapkavileiisseyr
xylTc2Nt        392 .....................................................................................
xylTc7Nt        386 .....................................................................................

AJ627182        481 rphfaliaqwkgleyhpiylegsyadppvvidrlssilrslgc
xylTc2Nt        472 h.........................m------------
xylTc7Nt        466 ............................e-----------
```

Figure 3

```
AJ627183          1 agtcagagagagaagaagatgaacaagaaaaagctgaaaattcttgtttctctcttcgctctcaactcaatcactctcta
xylTg1Nt          1 ----------------------------------------cac.....................................
xylTg3Nt          1 ----------------------------------------cac.....................................
xylTg14Nb         1 ----------------------------------------cac.....................................
xylTg19Nb         1 ----------------------------------------cac.....................................

AJ627183         81 tctctacttctcttcccaccctgatcacttccgccacaaatcccgccaaaaccacttt--------------------t
xylTg1Nt         42 ..................t.............t.........c.............cctaatacccaaaaccactat.
xylTg3Nt         42 ..........................................................--------------------.
xylTg14Nb        42 ............................ct..t.g........c...g.........--------------------.
xylTg19Nb        42 ........................---------.........c.............--------------------.

AJ627183        140 ccttgtcggaaaaccgccatcataatttccactcttcaatcacttctcaatattccaagccttggcctattttgccctcc
xylTg1Nt        122 ..c............a....g................tg.......c......a.............a............
xylTg3Nt        101 ................................................................................
xylTg14Nb       101 ...c...........a.............................c..........g.....................
xylTg19Nb        92 ..............a.................................................................

AJ627183        220 tacctcccttggtctcaaaaccctaatgttgtttggagatcgtgcgagggttacttcggtaatgggtttactctcaaagt
xylTg1Nt        202 ........c........g..t.........tc..t.....................................................
xylTg3Nt        181 ................................................................................
xylTg14Nb       181 ................................c.........a.......................t..............
xylTg19Nb       172 ................................c.....................................................

AJ627183        300 tgaccttctcaaaacttcgccggagtttcaccggaaattcggcgaaaacaccgtctccggcgacggcggatggtttaggt
xylTg1Nt        282 ...t.....................c......a.....................a.........................
xylTg3Nt        261 ................................................................................
xylTg14Nb       261 ...t.....................c..................................t...a.................
xylTg19Nb       252 ..............................................t..............t.....................

AJ627183        380 gtttttttcagtgagactttgcagagttcgatctgcgagggaggcgcaatacgaatgaatccggacgagattttgatgtct
xylTg1Nt        362 ................................t...........t..t.............................
xylTg3Nt        341 ................................................................................
xylTg14Nb       341 ....c...........................................................a...............
xylTg19Nb       332 ....................................................................t...........
```

Figure 3 (continued)

```
AJ627183        460 cgtggaggtgagaaattggagtcggttattggtaggagtgaagatgatgagctgcccgtgttcaaaaatggagcttttca
xylTg1Nt        442 ........c...............................................................
xylTg3Nt        421 ...............................................................
xylTg14Nb       421 ..................................................g......c........c.....
xylTg19Nb       412 ....................................a...................a.....................c..

AJ627183        540 gattaaagttactgataaactgaaaattgggaaaaaattagtggatgaaaaattcttgaataaatacttaccggaaggtg
xylTg1Nt        522 ..................................................a............................
xylTg3Nt        501 ..................................................a............................
xylTg14Nb       501 ........................t.........................c............................
xylTg19Nb       492 a...g..............................c........a.............................g.....

AJ627183        620 caatttcaaggcacactatgcgtgagttaattgactctattcagttggttggcgccgatgattttcactgttctgaggtt
xylTg1Nt        602 ........................a.....................a..............a.................
xylTg3Nt        581 ........................a.....................a..............a.................
xylTg14Nb       581 ..............................c.......................a.....................
xylTg19Nb       572 .g.....................................................a.................

AJ627183        700 agattttg--aa-ttttgtttgctctttaaattaaaggtttaaactttgtgaatgttggcagatatggaatacactaatg
xylTg1Nt        682 ........--..a.....c...a.................g.................................a.....
xylTg3Nt        661 ........--..a.....c...a.................g.................................a.....
xylTg14Nb       661 .......tga..-.............................g.........................a.......a.....
xylTg19Nb       652 ........--.t-a...a....a...........g......g........t.....................a.....

AJ627183        777 gattttgtttgatctgtttaatgaagattgtctagaacctcaatgttataaatatg-gtttggttgcttcattaattaaa
xylTg1Nt        760 ..........................................t.............-.....t.................
xylTg3Nt        739 ..........................................t.............-.....t.................
xylTg14Nb       740 ..a....c.........................g...........c.........tt.....t.................
xylTg19Nb       729 ....................................a.........c.........tt.....t.................

AJ627183        856 gagaattccttaatatcccgactagatgccagataacaccagttagttgacttttggattattggttgcatttcatttga
xylTg1Nt        839 ...c.........a...t.....................................................g.a..ttt........a.
xylTg3Nt        818 ...c.........a...t.....................................................g.a..ttt........a.
xylTg14Nb       820 ...--------.........a.....................................--cg.a.............a.
xylTg19Nb       809 ...--------.....................................................--.g..............a.
```

Figure 3 (continued)

```
AJ627183        936 tcagataaattgttcattcttaaatgtttcactaaagaattactcaagatttcagagtttatatgtaggtgtatgtattt
xylTg1Nt        919 ........ga.ag..........................tg...t........t..a...c.t..a.----.......
xylTg3Nt        898 ........ga.ag..........................tg...t........t..a...c.t..a.----.......
xylTg14Nb       890 .......tgg.ac.........................t...tg.........................----.......
xylTg19Nb       879 .......tgg.ac...........................tg.....................a....----.......

AJ627183       1016 ggaattctggatttggatctagtattgaatggattactgaacttgtactccccagtcatctggggaggagcaatagatca
xylTg1Nt        995 ......t................c.......g.a....c.................................c....t.
xylTg3Nt        974 ......t................c.......g.a....c.................................c....t.
xylTg14Nb       966 ...........................................................................c....t.
xylTg19Nb       955 ..........................................t..................a..................g

AJ627183       1096 aattcaagggttgaaaagtaatactgagtcagaaattaaccactttaacttggaaaacggtaaatgtatgtgttctaaga
xylTg1Nt       1075 ........................a...................---..g........t..............g.......
xylTg3Nt       1054 ........................a...................---..g........t..............g.......
xylTg14Nb      1046 .c.................................g..........c...........tt........g....g.......
xylTg19Nb      1035 ..................................................-.....................

AJ627183       1176 tggttattcctataacttttgatgtctaatatggagaaagtgagttgatttatgctttttccttttccctttattggtgt
xylTg1Nt       1152 ..a...c.....................c..........t.............c...a..............a.t.
xylTg3Nt       1131 ..a...c.....................c..........t.............c...a..............a.t.
xylTg14Nb      1126 ..---...a..c..........g......c..........t.............c...a..............a.t.
xylTg19Nb      1114 ..a.....................................................................a...

AJ627183       1256 tggtttttaaattctatcaattcctttgtttgattgctactcaaattgaaccttagacggagtagcaatagcaaaaagtg
xylTg1Nt       1232 .......c.....................................g.............................--.......
xylTg3Nt       1211 .......c.....................................g.............................--.......
xylTg14Nb      1203 ...c...................a.....................................................
xylTg19Nb      1194 ................................g.....................................................

AJ627183       1336 aag----gccattctttt-ctcctttcatctctttatttccgtttgacatacagaatatggtagcatctgtctgaagtgg
xylTg1Nt       1310 ...aaaa......-....-.....................t....t...c.........................c.a.
xylTg3Nt       1289 ...aaaa......-....-.....................t....t...c.........................c.a.
xylTg14Nb      1283 ...aaag......t....t..............a..........t...c..............a.....----------
xylTg19Nb      1274 ...aaag.a....t....-.....................................c..........c.........
```

Figure 3 (continued)

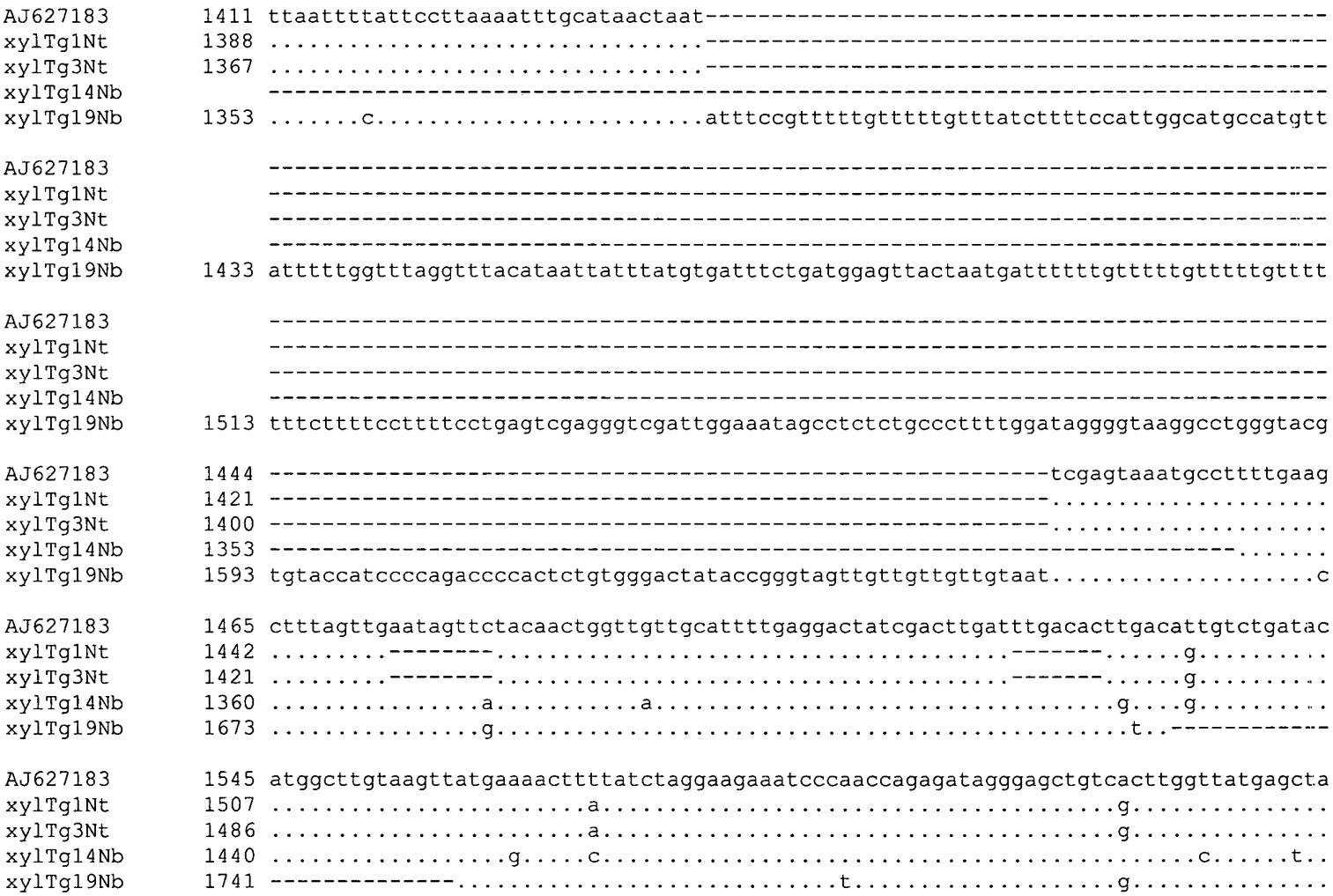

Figure 3 (continued)

```
AJ627183      1625 ctggctcaaagttcaagtttgaccagttaattttagatcttcaccaggataacatttagagtctaatcaaattctgaagc
xylTg1Nt      1587 .............g.........................--------------g.....................
xylTg3Nt      1566 .............g.........................--------------g.....................
xylTg14Nb     1520 .............g.......................c.....................................
xylTg19Nb     1807 ......t......tg.........ta.............................c......t....t.......c....

AJ627183      1705 agtattgtgcactaataagaggaacacatgaaggatgtagcactactaggttatgttaccttatttactaatgat---tg
xylTg1Nt      1652 ............................c.............g.c.............................---..
xylTg3Nt      1631 ............................c.............g.c.............................---..
xylTg14Nb     1600 ..................................................................a..gac..
xylTg19Nb     1887 ......t............g.............................c.........-.t..........t....---..

AJ627183      1782 acaaccagcttaaatgatgacaaatagtcttatatttgctttttcacattgctcatgactt-gggatatttccgaatcaa
xylTg1Nt      1729 .g.....................g......g.....-.....t.................t..........t.g.....
xylTg3Nt      1708 .g.....................g......g.....-.....t.................t..........t.g.....
xylTg14Nb     1680 .............t...........g.............c....-.................-..........t......g
xylTg19Nb     1963 .......................g.................t.................-.........tt.......

AJ627183      1861 catattttagttctttatgtacttaactacttatcaaaaaattatccctgctaga-------tgttagtgttcaagcaac
xylTg1Nt      1808 ...t..cc..........--------.............................gcctaga.................
xylTg3Nt      1787 ...t..cc..........--------.............................gcctaga.................
xylTg14Nb     1758 ...t...c..........--------.................................-------.................
xylTg19Nb     2042 ...t...cg.........--------.................................-------.................-

AJ627183      1934 catgctagcttttaaggaagctccttctttgattcatgccatctttccgaaatcgatgccttacgttactgtcatttttc
xylTg1Nt      1880 ..................................................t.............................
xylTg3Nt      1859 ..................................................t.............................
xylTg14Nb     1823 .........a.....c.......................g.........t....t.........................
xylTg19Nb     2106 ..........................................................------..........t.....

AJ627183      2014 taattttcatttcagtggattgaggagccgtcacttttgattacacgatttgagtatgcaaacctttccacacagttac
xylTg1Nt      1960 ..................................................................................
xylTg3Nt      1939 ..................................................................................
xylTg14Nb     1903 ..................................................................a....
xylTg19Nb     2180 ..................g.................................................................
```

Figure 3 (continued)

```
AJ627183      2094 cgattggtatagtgcatacgtggcatccagggttactggcttgcccagtcggccacatttggtttttgtagatggccatt
xylTg1Nt      2040 ....................................................................................
xylTg3Nt      2019 ....................................................................................
xylTg14Nb     1983 ........................g...........................................................
xylTg19Nb     2260 ....................c.................t................a.........................

AJ627183      2174 gtgaggtatgtttgaaagtattgataacgatggcatgcattgtactgtgttacggatgaaagaaatgaaaccagcaatta
xylTg1Nt      2120 ..................................................t.................t.........
xylTg3Nt      2099 ..................................................t.................t.........
xylTg14Nb     2063 ...........c.....................................c...t.............................
xylTg19Nb     2340 ..............c.....................................t.....................t......

AJ627183      2254 ttttctagcaggcaatgctcttgagatgcttgtgtcaaattggtcagacttaatcctgagtttccatttgtttcagcttt
xylTg1Nt      2200 ....................................................................................
xylTg3Nt      2179 ....................................................................................
xylTg14Nb     2143 ....................................................................................
xylTg19Nb     2420 ........t.............a.....................t...g........a...............g......

AJ627183      2334 ctgtttgactgactacaataattgtcccaattaggggtgtcaatggatattcgaaagccgactaaaccgaccgaaccgta
xylTg1Nt      2280 ......................----------------------------------------------------------
xylTg3Nt      2259 ......................----------------------------------------------------------
xylTg14Nb     2223 ....g................----------------------------------------------------------
xylTg19Nb     2500 ...................----------------------------------------------------------

AJ627183      2414 ccgtaccgatttttaggtttctttttaagaaaccgtaggttttatataaatctataaccgcaccgataattagggtagg
xylTg1Nt           ----------------------------------------------------------------------------
xylTg3Nt           ----------------------------------------------------------------------------
xylTg14Nb          ----------------------------------------------------------------------------
xylTg19Nb          ----------------------------------------------------------------------------

AJ627183      2494 ttttttatttttataaaaataagccgaaaaaataccgaaccgtaccgaataaattttacatgtggaaaatatatttattta
xylTg1Nt           ----------------------------------------------------------------------------
xylTg3Nt           ----------------------------------------------------------------------------
xylTg14Nb          ----------------------------------------------------------------------------
xylTg19Nb          ----------------------------------------------------------------------------
```

Figure 3 (continued)

```
AJ627183      2574 gtaagtttaaaaataataatgcattaaattttctttgggccatggaattatgaaaactattacaagccaacaagtaatta
xylTg1Nt           ----------------------------------------------------------------------------
xylTg3Nt           ----------------------------------------------------------------------------
xylTg14Nb          ----------------------------------------------------------------------------
xylTg19Nb          ----------------------------------------------------------------------------

AJ627183      2654 gactcaaaatactaattcctaaaacctattatgttacttctacttaaactaagttatttcaagtatctttattagcaaga
xylTg1Nt           ----------------------------------------------------------------------------
xylTg3Nt           ----------------------------------------------------------------------------
xylTg14Nb          ----------------------------------------------------------------------------
xylTg19Nb          ----------------------------------------------------------------------------

AJ627183      2734 cacaaagtattctagcgattatgagcaaactacaatgtattgaatatgtttcctttcatatattttagatttatcttttt
xylTg1Nt           ----------------------------------------------------------------------------
xylTg3Nt           ----------------------------------------------------------------------------
xylTg14Nb          ----------------------------------------------------------------------------
xylTg19Nb          ----------------------------------------------------------------------------

AJ627183      2814 gaatatttaatcttctatagactctattcttgagtcccagcttggtatatctttcaactcgtgtgatttatattttcttt
xylTg1Nt           ----------------------------------------------------------------------------
xylTg3Nt           ----------------------------------------------------------------------------
xylTg14Nb          ----------------------------------------------------------------------------
xylTg19Nb          ----------------------------------------------------------------------------

AJ627183      2894 gcctttgtttgatttcttttacgttgttgtagaatagtcgatggatctatactctagccatctttcttttttcttttttta
xylTg1Nt           ----------------------------------------------------------------------------
xylTg3Nt           ----------------------------------------------------------------------------
xylTg14Nb          ----------------------------------------------------------------------------
xylTg19Nb          ----------------------------------------------------------------------------

AJ627183      2974 attcatcacctttaaacagtaaaaatgtctaaagaatttttctaagtcctataaaagaacgtatgttattgcattctac
xylTg1Nt           ----------------------------------------------------------------------------
xylTg3Nt           ----------------------------------------------------------------------------
xylTg14Nb          ----------------------------------------------------------------------------
xylTg19Nb          ----------------------------------------------------------------------------
```

Figure 3 (continued)

```
AJ627183      3054 ttctactggtgaattttacatgatattaaaaaattaaccgaaccttaccgctaccgaagagaaaccgacatgattgggac
xylTg1Nt           ----------------------------------------------------------------------------------
xylTg3Nt           ----------------------------------------------------------------------------------
xylTg14Nb          ----------------------------------------------------------------------------------
xylTg19Nb          ----------------------------------------------------------------------------------

AJ627183      3134 ggtttcgaaaagtctaattttggttatacataatagaataaccgaaaaattggtatggtacaaattttataaaataaccg
xylTg1Nt           ----------------------------------------------------------------------------------
xylTg3Nt           ----------------------------------------------------------------------------------
xylTg14Nb          ----------------------------------------------------------------------------------
xylTg19Nb          ----------------------------------------------------------------------------------

AJ627183      3214 gccgaaccgaaccattgacacccctagtcccaatacctagttgttgcagtttgctcattcttacttctatttacgt--ca
xylTg1Nt      2303 --------------------------.....................c.g...g.........................gt..
xylTg3Nt      2282 --------------------------.....................c.g...g.........................gt..
xylTg14Nb     2246 --------------------------.....g.......a...........g...........t.............gt..
xylTg19Nb     2520 ------------------------..t.....................g...g.........................gt..

AJ627183      3292 ctgtttctctgaatggtccctttgtggtgaaaagagcttttgctatgtagaaaaactagcaatgatttcatagctgaaac
xylTg1Nt      2357 .................................t............a..............a........tt...gg..
xylTg3Nt      2336 .................................t............a..............a........tt...gg..
xylTg14Nb     2300 ................-.......................a......a............a........tt...g...
xylTg19Nb     2577 ..........................................................a........tt...g.g.

AJ627183      3372 aatttatttttaccttacatcatgtcttataaaattgcttctaactgtatactttaattcttggagagatgctttcatgt
xylTg1Nt      2437 ....................c....c.............ct.t..c.........................a....
xylTg3Nt      2416 ....................c....c.............ct.t..c.........................a....
xylTg14Nb     2379 .....c..............c..g.c.....................................................
xylTg19Nb     2657 ..c.................c....c.....................................................

AJ627183      3452 gaagaaagttctttcactccact-----------------------------------------------------actgca
xylTg1Nt      2517 ....................tttaattcttggagagatgctttaatgtgaagaaagttctttcactccact....t.
xylTg3Nt      2496 ....................tttaattcttggagagatgctttaatgtgaagaaagttctttcactccact....t.
xylTg14Nb     2459 ....................-------------------------------------------------------......
xylTg19Nb     2737 ...t................-------------------------------------------------------......
```

Figure 3 (continued)

```
AJ627183      3481 agcttgccgtatgaattttacttggccatattgtggccgtgctttgatttatcttcaaattcattttcttcatatagttc
xylTg1Nt      2597 ......t.c.....................g..g....t.............................g..a...
xylTg3Nt      2576 ......t.c.....................g..g....t.............................g..a...
xylTg14Nb     2488 ......t.c.....................g......t.............................g......
xylTg19Nb     2766 ......t.c.....a.............c..g......t........g.....................g......

AJ627183      3561 tttcgagtaattctttttttcctcttttctgtttga-aaaaaattcagacacaattggaggaaacatggaaagcacttttt
xylTg1Nt      2677 ...t........-......t..g...........gg.....................................c...
xylTg3Nt      2656 ...t........-......t..g...........gg.....................................c...
xylTg14Nb     2568 ............-.....................-.....t..................................
xylTg19Nb     2846 ..........-.a..........--..........-.....................................

AJ627183      3640 tcaagcctcacttatgctaagaactttagtggcccagtttgtttccgtcatgccgccctctcgcctttgggatatgaaac
xylTg1Nt      2756 ...................................................c....tt.....................
xylTg3Nt      2735 ...................................................c....tt.....................
xylTg14Nb     2646 .....................................................t.....................
xylTg19Nb     2922 .....................................................t.t...........a...........

AJ627183      3720 tgccctgtttaagggactgtcagaaactatagattgtaatggagcttctgcccatgatttgtggcaaaatcctgatgata
xylTg1Nt      2836 ..................a..................................................................
xylTg3Nt      2815 ..................a..................................................................
xylTg14Nb     2726 ....................................................t.............................
xylTg19Nb     3002 ....................................................t.................g...........

AJ627183      3800 agaaaactgcacggttgtccgagtttggggagatgattagggcagcctttagatttcctgtggatagacagaacatccca
xylTg1Nt      2916 ...g.................................c............g.................................
xylTg3Nt      2895 ...g.................................c............g.................................
xylTg14Nb     2806 ..............a.....................c............g..........t.....................
xylTg19Nb     3082 .a..................................c............g.................................

AJ627183      3880 aggacagtcacaggccctaatgtcctctttgttagacgtgaggattatttagctcacccacgtcatggtggaaaggtaca
xylTg1Nt      2996 ...............................................................................
xylTg3Nt      2975 ...............................................................................
xylTg14Nb     2886 ...............................................................................
xylTg19Nb     3162 ...............................................................................
```

Figure 3 (continued)

```
AJ627183      3960 gtctaggcttagcaatgaagagcaagtatttgattccataaagagctgggccttgaaccactcggagtgcaaattaaatg
xylTg1Nt      3076 ....................................................................................
xylTg3Nt      3055 ....................................................................................
xylTg14Nb     2966 ..........................................................a.........................
xylTg19Nb     3242 ........................t.....................................................t..........

AJ627183      4040 taattaacggattgtttgcccacatgtccatgaaagagcaagttcgagcaatccaagatgcttctgtcatagttggtgct
xylTg1Nt      3156 ..........................................................a........t..c..c...
xylTg3Nt      3135 ..........................................................a........t.........
xylTg14Nb     3046 ......gt..................................................................t.........
xylTg19Nb     3322 ....................................................................................

AJ627183      4120 catggagcaggtctaactcacatagtttctgcagcaccaaaagctgtaatactagaaattataagcagcgaatataggcg
xylTg1Nt      3236 ....................................................................................
xylTg3Nt      3215 ....................................................................................
xylTg14Nb     3126 ................c...................................................................
xylTg19Nb     3402 ....................................................................................

AJ627183      4200 cccccattttgctctgattgcacaatggaaaggattggagtaccatcccatatatttggaggggtcttatgcggatcctc
xylTg1Nt      3316 ....................................................................................
xylTg3Nt      3295 ....................................................................................
xylTg14Nb     3206 ....................t...............................................................
xylTg19Nb     3482 ....................................................................................

AJ627183      4280 cagttgtgatcgacaagctcagcagcattttgaggagtcttgggtgctaaatctgctcgacagtttagttcgtcttttct
xylTg1Nt      3396 ............------------------------------------------------------------------
xylTg3Nt      3375 ............------------------------------------------------------------------
xylTg14Nb     3286 ............------------------------------------------------------------------
xylTg19Nb     3562 ............------------------------------------------------------------------

AJ627183      4360 ctaaaagactgggaaggatagaggaattcggggttctggaacctggagcctgggaattgtgtaaaatatgtttcacacgc
xylTg1Nt           --------------------------------------------------------------------------------
xylTg3Nt           --------------------------------------------------------------------------------
xylTg14Nb          --------------------------------------------------------------------------------
xylTg19Nb          --------------------------------------------------------------------------------
```

Figure 3 (continued)

```
AJ627183      4440 agttctatagtcaattgctgcaatctggtgttcataagcttggaaatttccagcagctactaacttattagcccactctg
xylTg1Nt           ------------------------------------------------------------------------------
xylTg3Nt           ------------------------------------------------------------------------------
xylTg14Nb          ------------------------------------------------------------------------------
xylTg19Nb          ------------------------------------------------------------------------------

AJ627183      4520 actcagttatggactaccagagcaatcatatcaaatgggagcatggaatcctgattgtggaatggtgagctcattgaaga
xylTg1Nt           ------------------------------------------------------------------------------
xylTg3Nt           ------------------------------------------------------------------------------
xylTg14Nb          ------------------------------------------------------------------------------
xylTg19Nb          ------------------------------------------------------------------------------

AJ627183      4600 gcatattctttatggtgttgaagattacagttgacgagtaacacgtgtatgtgaaagattaggttgttacactttcttgc
xylTg1Nt           ------------------------------------------------------------------------------
xylTg3Nt           ------------------------------------------------------------------------------
xylTg14Nb          ------------------------------------------------------------------------------
xylTg19Nb          ------------------------------------------------------------------------------

AJ627183      4680 aattcattgtcaattgtttttcgtcattcttattaatgatcataggataagaacatgagaaaaccatccatgttctctgt
xylTg1Nt           ------------------------------------------------------------------------------
xylTg3Nt           ------------------------------------------------------------------------------
xylTg14Nb          ------------------------------------------------------------------------------
xylTg19Nb          ------------------------------------------------------------------------------

AJ627183      4760 tgttttcccatcaatctggccaccctctttcctctttatgtagagatgatttcaacagagtttgttttgtagttgtaata
xylTg1Nt           ------------------------------------------------------------------------------
xylTg3Nt           ------------------------------------------------------------------------------
xylTg14Nb          ------------------------------------------------------------------------------
xylTg19Nb          ------------------------------------------------------------------------------

AJ627183      4840 cttgtactcacagttactgttttgcattcatcccatcagatgtcgaagaagcagattaacaagaacgtcagtatgatgtt
xylTg1Nt           ------------------------------------------------------------------------------
xylTg3Nt           ------------------------------------------------------------------------------
xylTg14Nb          ------------------------------------------------------------------------------
xylTg19Nb          ------------------------------------------------------------------------------
```

Figure 3 (continued)

```
AJ627183    4920 tcagtgaatatatggttgtaacttgtaaccaaacaaaagaaatgagactttgac
xylTg1Nt         ------------------------------------------------------
xylTg3Nt         ------------------------------------------------------
xylTg14Nb        ------------------------------------------------------
xylTg19Nb        ------------------------------------------------------
```

Figure 4


AJ627183 1 mnkkklkilvslfalnsitlylyfsshpdhfrhksrqnhf-------slsenrhhnfhssitsqyskpwpilpsylpwsq
xylTg1Nt 1 ---------.....s.....p....pntqnhy.....h.d.....v....t.....
xylTg3Nt 1 ---------.....-------.....
xylTg14Nb 1 ---------.....s.r..p....-------.s...h.....r.....
xylTg19Nb 1 -------h.....---...p....-------.....h.....
AJ627183 74 npnvvwrscegyfgngftlkvdllktspefhrkfgentvsgdggwfrcffsetlqssiceggairmnpdeilmsrggekl
xylTg1Nt 72 ....sl.....l.q.....
xylTg3Nt 65 .....
xylTg14Nb 65 ....a.....l.....f.....
xylTg19Nb 64 ....a.....d.....d.....
AJ627183 154 esvigrseddelpvfkngafqikvtdklkigkklvdekflnkylpegaisrhtmrelidsiqlvgaddfhcsewieepsl
xylTg1Nt 152 .....i.....e.....
xylTg3Nt 145 .....i.....e.....
xylTg14Nb 145 .....v.a..t.....f.....n.....n.....
xylTg19Nb 144 .....n.....m.....e.....k.....g.....e.....v.....
AJ627183 234 litrfeyanlfhtvtdwysayvasrvtglpsrphlvfvdghcetqleetwkalfssltyaknfsgpvcfrhaalsplgye
xylTg1Nt 232 .....v.....
xylTg3Nt 225 .....v.....
xylTg14Nb 225 .....i.....v.....
xylTg19Nb 224 .....a.....n.....v.....
AJ627183 314 talfkglsetidcngasahdlwqnpddkktarlsefgemiraafrfpvdrqniprtvtgpnvlfvrredylahprhggkv
xylTg1Nt 312 .....t.....r.....g.....
xylTg3Nt 305 .....t.....r.....g.....
xylTg14Nb 305 .....g.....
xylTg19Nb 304 .....k.....g.....
AJ627183 394 qsrlsneeqvfdsikswalnhsecklnvinglfahmsmkeqvraiqdasvivgahgaglthivsaapkavileiisseyr
xylTg1Nt 392 .....
xylTg3Nt 385 .....
xylTg14Nb 385 .....s.....
xylTg19Nb 384 .....l.....

Figure 4 (continued)

```
AJ627183      474 rphfaliaqwkgleyhpiylegsyadppvvidklssilrslgc
xylTg1Nt      472 ..........................----------
xylTg3Nt      465 ..........................----------
xylTg14Nb     465 ..........................----------
xylTg19Nb     464 ..........................----------
```