CHAPTER 2

Sialoglycans and genetically engineered plants

1 Introduction

N-glycosylation proteins enable proper protein folding and provides stability to the protein, efficient protein targeting for activity of lysosomal enzymes, protein-protein or protein-carbohydrate interaction, effector functions and biological activity of proteins, and control of protein half-life (Fig. 1).

Plants are being engineered to develop large quantities of biopharmaceutical proteins (Table 1) in a cost-effective manner and several technical, veterinary, and pharmaceutical proteins made in plants have been successfully made available commercially [2]. Since plant *N*-glycosylation pathway differs at some stages with the human *N*-glycosylation pathway, it is important to manipulate the plant *N*-glycosylation pathway to render them more appropriate expression systems for human *N*-glycosylated proteins of desired properties.

Solanaceous species under genus *Nicotiana*, such as *Nicotiana tabacum* (tobacco) and *Nicotiana benthamiana* (*N. benthamiana*, Fig. 2) have been extensively exploited in molecular farming due to the following advantages: (i) ease of cultivation, (ii) high biomass, (iii) genetic tools for trait manipulation, (iv) application of new plant breeding techniques (CRISPR/Cas9); and (v) nonfood status of the plant minimizing chances of contamination, (vi) possibility of natural insertion in the RNA-dependent RNA polymerase 1 gene [1, 3], which leads to a reduced level of gene silencing [1, 4].

Although the recombinant proteins of human origin are generated in plants with proper folding and it is possible to assemble complex proteins within the plant machinery, conventional expression systems for the production of recombinant biopharmaceutical proteins suffers from the limitation of proper synthesis of glycan structure in glycoconjugated molecules leading to the production of aberrant mixture of glycoforms that bear no resemblance to human glycans or are important from the point of view of therapy.

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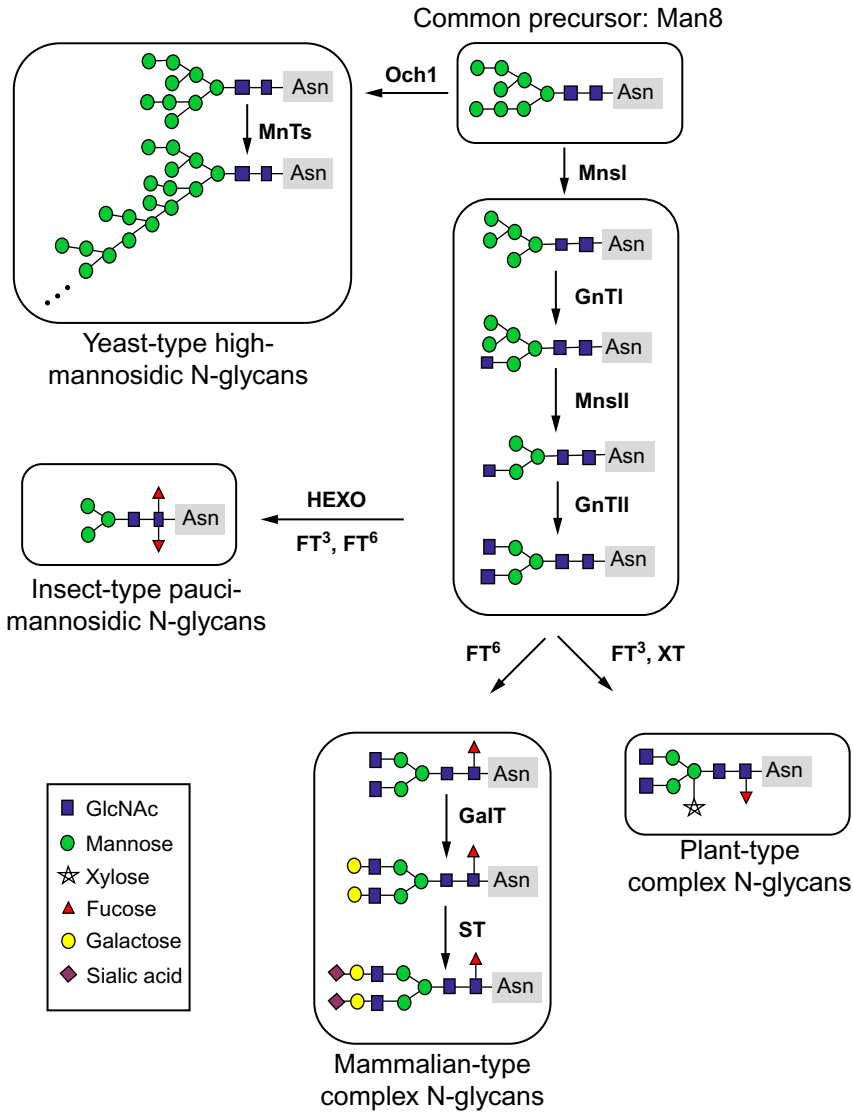

Fig. 1 N-glycosylation pathway represented from common precursor in plant human, yeast, and insect cells. The common endoplasmic reticulum (ER)-resident oligosaccharide precursor Man8 acts as initiating point for further modifications in the Golgi apparatus. Och1: a1,6-mannosyltransferase; MnTs: mannosyltransferases; Mns: mannosidase; GnT: *N*-acetylglucosaminyltransferase; GalT: a1,4-galactosyltransferase; ST: a2,6-sialyltransferase; HEXO: hexosaminidase (*N*-acetylglucosaminidase); XT: β 1,2-xylosyltransferase; and FT: core fucosyltransferase. Fucose can be transferred in a1,3-linkage (typical of plant) and a1,6-linkage (typical of mammal). *(Reproduced with permission from Loos A, Steinkellner H IgG-Fc glycoengineering in non-mammalian expression hosts, Arch Biochem Biophys 2012;526:167–173.)*

Table 1 Plant-made pharmaceuticals and clinical trials

Product	Host	Application	Clinical trial	Status	Sponsor
Taliglucerase alfa; Recombinant glucocerebrosidase (prGCD)	Carrot cell culture	Gaucher disease	NCT00376168	Phase 3 completed (2012); FDA approved (2012)	Protalix, Karmiel, Israel
ZMapp	Tobacco	Ebola Virus	NCT02363322	Phase 1 and 2 (2015)	National Institute of Allergy and Infectious Diseases (NIAID), Bethesda, MD, USA
PRX-102	Tobacco cell culture	Fabry Disease	NCT01769001	Phase 1 and 2 (2014)	Protalix, Karmiel, Israel
VaccinePfs25 VLP	Tobacco	Malaria	NCT02013687	Phase 1 (2015)	Center for Molecular Biotechnology, Plymouth, MI, USA
Vaccine Recombinant protective antigen	Tobacco	Anthrax	NCT02239172	Phase 1 (2014)	Center for Molecular Biotechnology, Plymouth, MI, USA
HAI-05	Tobacco	H5N1 Vaccine	NCT01250795	Phase 1 (2011)	Center for Molecular Biotechnology, Plymouth, MI, USA
Recombinant human intrinsic factor	*Arabidopsis thaliana* (*A. thaliana*)	Vitamin B12 deficiency	NCT00279552	Phase 2 Completed (2006)	University in Aarhus, Aarhus, Denmark
H5-VLP + GLA-AF Vaccine	Tobacco	Influenza A Subtype H5N1 Infection	NCT01657929	Phase 1 Completed (2014)	Infectious Disease Research Institute, Seattle, WA, USA
P2G12 Antibody	Tobacco	HIV	NCT01403792	Phase 1 Completed (2011)	University of Surrey, Guildford, UK

Fig. 2 *Nicotiana* sp. *(Reproduced from Wikipedia Source Wikipedia: Joachim Müllerchen—Own work Multi-license with GFDL and Creative Commons CC-BY 2.5 https://commons.wikimedia.org/wiki/File:Tabak_9290019.JPG.)*

Thus the synthesis of glycans resembling human glycan structure and its huge complexity remain a major challenge in glycoengineering of plant cells [6]. This is all more challenging due to the complexity and diversity of the glycans in different therapeutic recombinant proteins.

Targeted manipulation of the plant *N*-glycosylation pathway has enabled the production of human-like oligosaccharides and enabled the generation of functional and effective biopharmaceuticals. In the recent years plant revealing a simple *N*-glycosylation pathway but lacking the *O*-glycosylation pathway have been reported to be better and potential glycan expression systems over the conventional ones. Plant systems are being used as effective expression systems of complex sialoglycans and *N*-glycans and different strategies are being used for the expression of complex therapeutic sialylated glycoforms in plant systems.

2 *N*-glycosylation in plants

Recently different posttranslational modifications (PTMs) have been reported for peptide maturation and activation, including proteolytic processing, tyrosine sulfation, proline hydroxylation, and hydroxyproline glycosylation [7] in plants. While glycan epitopes of human complex *N*-glycans are often targets of lectins important for cell-cell communication, the role of plant *N*-glycans finds importance in protein folding, and other biological functions including salt stress responses, cellulose biosynthesis, microtubule association, and biogenesis of several receptor-like kinases [8].

N-glycosylation is a major post-translational modification PTM in eukaryotes and is important in maintaining cell viability, where the attached *core N*-glycans enables proper protein folding of secreted glycoproteins and membrane proteins in the endoplasmic reticulum (ER).

Although the studies of *N*-glycosylation in human is extensive, the knowledge in plants is restricted due to limited *N*-linked glycan and mutant phenotypes, limited methods to modify and target *N*-glycans at specific sites, and limited understanding of protein dynamics within secretory system. Glycoproteins move from the ER to the Golgi apparatus, where the *N*-glycan moieties undergo further maturation or may exit the ER via an alternative route to vacuoles retaining high-mannose *N*-glycan structures bypassing the modifications in the Golgi.

In eukaryotes, *N*-glycans processing is initiated in the ER where the precursor Glc3Man9GlcNAc2 (Man9) is converted to Man8GlcNAc2 (Man8) and processing of Man8 in Golgi leads to the formation of complex *N*-glycans (Fig. 1). *N*-glycan processing is identical in plants and mammals till the formation of vital intermediate GlcNAc2Man3GlcNAc2 (GnGn). In mammals, GnGn oligosaccharides enables diversification of *N*-glycosylation but in plants, the GnGn structures are arranged with 1,2-xylose and core 1,3-fucose residues (GnGnXF3). Although in mammals core fucosylation occurs in 1,6-linkage, the fucose residues in plants (Fig. 3) are in 1,3-linkage. Plant cells extend the GnGnXF3 by attaching 1,3-galatose and 1,4-fucose to form Lewis-a epitopes (Lea). Plants reveal formation of paucimannosidic structures due to the removal of terminal GlcNAc residues from GnGnXF3 by endogenous hexosaminidases similar to insects. As compared to human complex *N*-glycans, *N*-glycans of the plant systems lack sialic acid but contain *core* α1,3-fucose (Fuc) and β1,2-xylose (Xyl) modifications, and may contain terminal Lewis-a epitopes (β1,3-galactose (Gal) and α1,4-Fuc

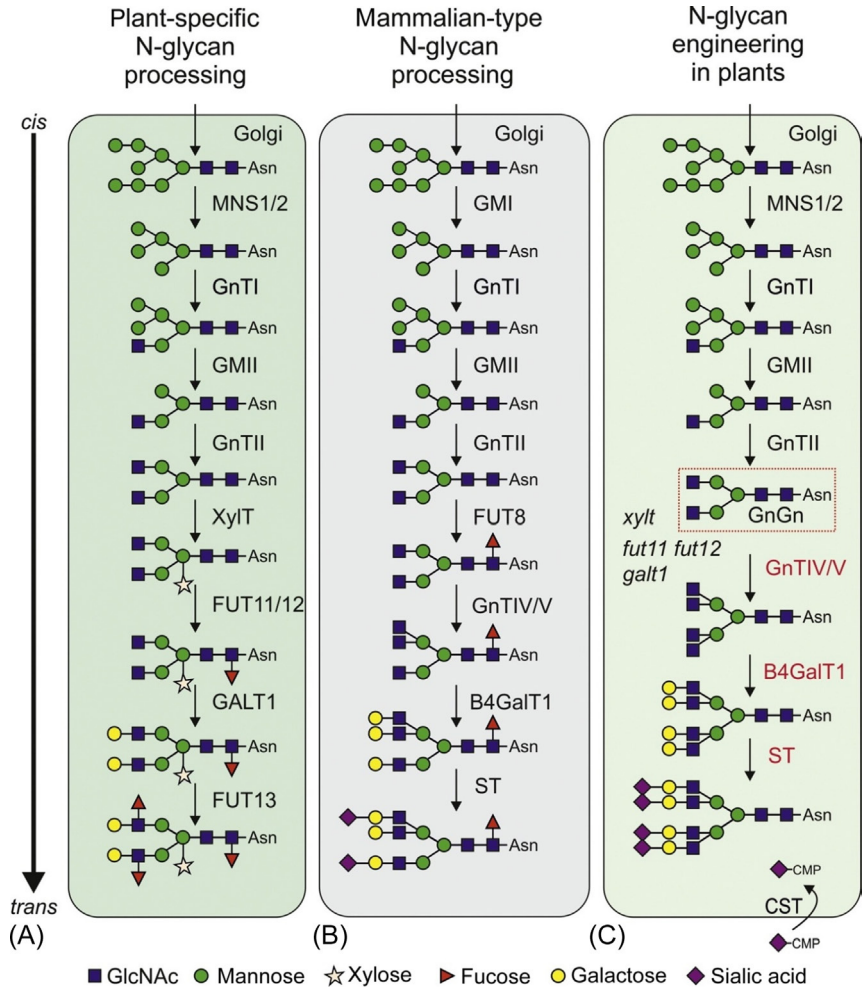

Fig. 3 (A) Schematic overview of complex *N*-glycan processing in plants. Golgi-α-mannosidase I (MNS1/2), *N*-acetylglucosaminyltransferase I (GnTI), Golgi-α-mannosidase II (GMII), *N*-acetylglucosaminyltransferase II (GnTII), β1,2-xylosyltransferase (XylT), core α1,3-fucosyltransferase (FUT11/12), β1,3-galactosyltransferase (GALT1), and α1,4-fucosyltransferase (FUT13). (B) Representative view of *N*-glycan processing in mammalian cells. Golgi α-mannosidase I (GMI), core α1,6-fucosyltransferase (FUT8), *N*-acetylglucosaminyltransferase IV (GnTIV) and V (GnTV), β1,4-galactosyltransferase (B4GalT1), and α2,6-sialyltransferases (ST). (C) Optimized *N*-glycan engineering approach: generation of *xylt*, *fut11*, *fut12*, and *galt1* knockouts results in the formation of GnGn structure which serves as acceptor for GnTIV, GnTV, B4GalT1, and ST resulting in fully processed complex *N*-glycans. Sialylation in plants requires the co-expression of the Golgi CMP-sialic acid transporter (CST) and proteins for CMP-sialic acid biosynthesis. *(Reproduced with permission from open access article under a Creative Commons Licence: Schoberer J Strasser R, Plant glyco-biotechnology.Semin Cell Dev Biol. 2018 80:133-141.)*

linked to terminal *N*-acetylglucosamine, GlcNac (Fig. 3). Human complex *N*-glycans are often sialylated containing different epitopes, including Lewis x, *N*-acetyllactosamine (LacNAc), and *N,N′*-di-*N*-acetyllactosediamine (LacDiNAc).

Although the plants and mammals reveal differences in the *N*-glycan structures they share high degree of homology in the secretory pathway.

3 Sialylation and recombinant proteins produced in plants

Glycoengineering aims at the production of recombinant glycoproteins with a defined glycosylation profile, in order to study the impact of glycosylation and for the production of therapeutic agents. The plant expression systems are being designed to generate therapeutically important glycoproteins. Plant systems find importance as they are biologically safe, cost effective, and convenient. However, as plant *N*-glycosylation pathway differs in many aspects as compared to human *N*-glycosylation, modification of *N*-glycosylation pathway in plants is needed to avoid immunological challenges and get humanized authentic *N*-glycosylated molecules.

Plants reveal highly conserved secretory pathway with folding, assembly, and posttranslational modifications of proteins similar to the mammals. Animal sialyltransferases (STs) consist of four conserved motifs, namely large (L), small (S), very small (VS), and motif III. Although sialic acid has not been detected in plants, three orthologues containing sequences similar to the ST motifs have been identified in *Arabidopsis thaliana* L. The At3g48820 gene with gene id 824,043 codes for a Golgi resident protein but lacks the ability to transfer sialic acid to asialofetuin or Galβ1,3GalNAc and Galβ1,4GlcNAc oligosaccharide acceptors [10]. Strategies to produce humanized therapeutic glycoproteins in plants involves (i) retaining of the recombinant glycoproteins in ER, where *N*-glycans undergo modification, (ii) inhibiting the plant endogenous Golgi glycosyltransferase, and (iii) adding new glycosyltransferase from mammals.

Different approaches have been used to modify the *N*-glycosylation pathway in different plant species, using T-DNA insertion mutants [11], RNA interference (RNAi) [12–14], chemical mutagenesis [15], and targeted nuclease [16–18] approaches. *N. benthamiana* finds importance in molecular farming as the transient expression of proteins is fast and yields antibodies [19] by different transient expression systems, including the MagnICON system [20], the pEAQ vector [21], and the pTRA vector [22]. Zinc finger nucleases (ZFNs) [23] transcription activator-like effector nucleases

(TALENs) [24] have enabled easy knockout of multiple genes. In *N. benthamiana*, the two *XylT* genes and two of the five *FucT* genes were knocked out with TALENs to completely eliminate the β-1,2-xylosyltransferase activity and reduce core α-1,3-fucosyltransferase activity by 60%. CRISPR/Cas9 system has been used to knockout two β-1,2-xylosyltransferase and four α-1,3-fucosyltransferase genes in *N. benthamiana* [25].

Sia and polysialic acid (polySia) play a vital role in biological functions and therapeutic use. Expression system in plants has been designed with multigene vectors enabling the controlled in vivo synthesis of sialylated structures in the human sialylation pathway (Fig. 4) that sialylate glycoproteins in α2,6- or α2,3-linkage and transient coexpression of human α2,8-polysialyltransferases lead to the production of active and functional polySia structures [26].

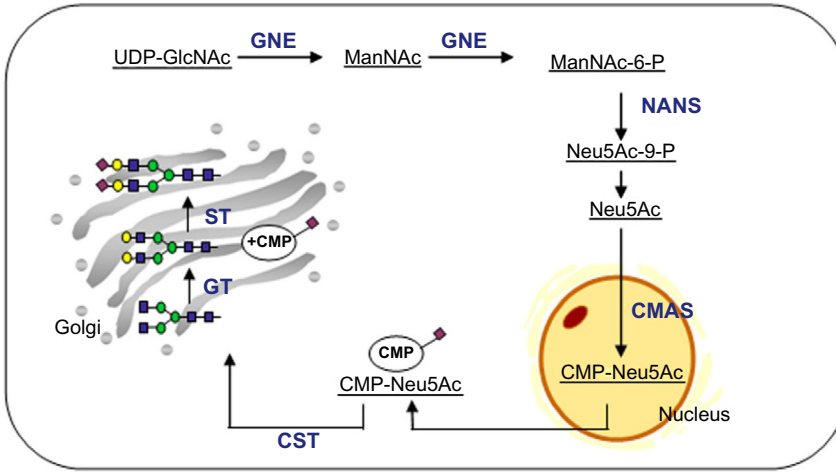

Fig. 4 Strategy to engineer human sialylation pathway in plants using the endogenously present metabolite UDP-GlcNAc. Enzymes involved are: UDP-*N*-acetylglucosamine 2-epimerase/*N*-acetylmannosamine-kinase (GNE), *N*-acetylneuraminic acid phosphate-synthase (NANS), CMP-sialic acid (Neu5Ac) synthetase (CMAS), CMP-Neu5Ac transporter (CST), β1,4-galactosyltransfease (GT), and α2,6-sialyltransferase (ST). In planta protein sialylation was achieved by the coordinated expression and correct subcellular deposition of genes/proteins for (i) biosynthesis (GNE, NANS), (ii) activation (CMAS), (iii) transport (CST), and (iv) transfer of Neu5Ac to terminal galactose (ST). *(Reproduced with permission from open access article under a Creative Commons License: Loos A, Steinkellner H IgG-Fc glycoengineering in non-mammalian expression hosts, Arch Biochem Biophys 2012;526:167–173.)*

4 Fc glycoengineering in plants

The conserved secretory pathway between plants and mammals enable the production of IgGs and IgAs efficiently. En block transfer of the Glc3Man9GlcNAc2 precursor onto the growing protein and subsequent trimming in ER and cis/medial-Golgi compartments reveal similarities between mammals and plants up to the synthesis of GnGn structures (Fig. 1). After this, in mammals, GnGn structures undergo intensive elongation/modification processes unlike in plants, which add xylose in β1,2-position to the innermost mannose residue and fucose in α1,3-position to the innermost GlcNAc residue of the GnGn core oligosaccharide (Fig. 1) which are absent in mammalian cells. Monoclonal antibodies (mAbs) in plants exhibit a N-glycosylation profile with a single dominant oligosaccharide structure, GnGnXF3.

The humanization of the plant pathways were thoroughly investigated by Palacpac et al. [27] and Bakker et al. [28]. They overexpressed the human β1,4-glacatosyltransferase (GalT) in tobacco plants to elongate the plant-typical GnGnXF3 by β1,4-galactose leading to the formation of galactosylated structures and drastically reduced the degree of xylosylation and fucosylation. Nut production of mAbs (mAbs) suffered from challenges of formation of unexpected glycoforms and incompletely processed and hybrid structures [29, 30].

Mutants lacking plant-specific β1,2-xylose and core α1,3-fucose achieved by the elimination of endogenous enzymes, β1,2-xylosyltransferase (XT) and core α1,3-fucosyltransferase (FT3, Fig. 2) by knockdown and knockout approaches for the respective genes, and generated mutant plant lines of *A. thaliana*, *Lemna minor*, *N. benthamian*, moss *Physcomitrella patens*, DXT/FT plants (*N. benthamiana* glycosylation mutants lack plant-specific core β1,2-xylose and α1,3-fucose residues) were generated and found importance in the production of different mAbs and therapeutics. A schematic diagram of Fc glycoengineering is represented in Fig. 5.

Fc-*N*-glycosylation profiles of these mAbs achieved by the elimination of β1,2-xylose and core α1,3-fucose leading to the synthesis of human-type structures containing dominant GnGn with no detectable β1,2-xylose or α1,3-fucose residues revealed unaltered antigen binding and complement-dependent cytotoxicity CDC activity and enhanced antibody-dependent cell-mediated cytotoxicity ADCC, effector functions of antibody. This also enabled the generation of increased galactosylation, sialylation, branching, bisecting GlcNAc, or fucosylation.

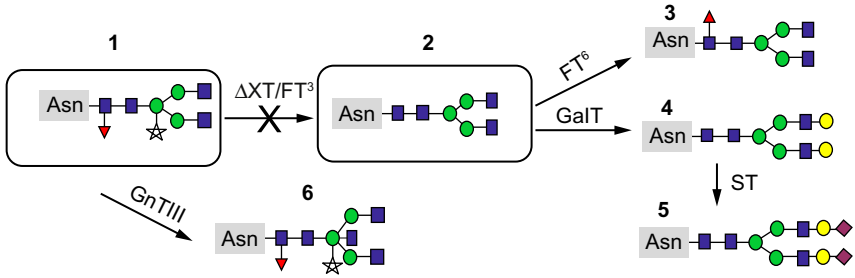

Fig. 5 Fc-glycoengineering in plants. Overview of mAb glycoforms generated in glycoengineered *N. benthamiana*. IgG N-glycans generated (1) in Wild type plants: GnGnXF3 [14]; (2) in DXT/FT3 plants: GnGn [14]; (3) in DXT/FT + FT6: GnGnF6 [31]; (4) in DXTFT + GalT: AA [32, 33], (5) in DXT/FT along with six mammalian genes of the mammalian sialic acid pathway: NaNa [29]; and (6) in Wild type + GnTIII: GnGnXF3bi [33]. FT6: a1,6-fucosyltransferase, GalT: b1,4-galactosyltransferase, ST: a2,6-sialyltransferase, and GnTIII: *N*-acetylglucosaminyltransferase III (1). *(Figure and legend reproduced with permission from open access article under a Creative Commons License: Loos A, Steinkellner H. IgG-fc glycoengineering in non-mammalian expression hosts. Arch Biochem Biophys 2012;526:167–73.)*

GalT when targeted to a late Golgi compartment significantly improved β1,4-galactosylation in DXT/FT, transgenic plants. mAbs produced in such glycoengineered plants exhibited a single dominant Fc-*N*-glycan, digalactosylated AA structures which is predominant in serum IgG and mAbs as against HIV produced in these glycoengineered plants which exhibited improved anti-viral activity. GlcNAc bound in b1,4-position to the innermost mannose residue called bisecting GlcNAc is reported to enhance ADCC activity of mAb CAMPATH-1H, glycoengineered Rituxan and Herceptin with increased bisecting structures due to decreased 1,6-fucosylation caused by the blocking of the fucosyltransferase.

Contrasting reports exist that in CHO cells the overexpression of *N*-acetylglucosaminyltransferase III (GnTIII) done with the hypothesis to increase bisecting GlcNAc, produced typical hybrid structures instead with significantly reduced core-fucose content.

In the DXT/FT mutant lacking plant-specific core modifications, less of bisecting glycoforms were synthesized as compared to Wild type plants. Glycomodified DXT/FT plants produced mammalian-type core α1,6-fucosylation by overexpressing core α1,6-fucosyltransferase, generating mAbs with and without fucose with identical *N*-glycosylation.

Plant based antibody 2G12 batches exhibited glycosylation profiles containing a predominant *N*-glycan structure, and GnGnXF3, GnGnF6, GnGn, and digalactosylated AA structures with binding similar to FccRI,

FccRIIa, and FccRIIb. 2G12 glycoforms lacking core fucose mediated antiviral activity against various lentiviruses including HIV-1.

The most complex step of human *N*-glycosylation is terminal sialylation and difficult to accomplish in plants as they lack the enzyme cascades. But in planta the sialylation of mAbs has recently become possible [12, 34] by the introduction of enzymes of the mammalian pathway into plants, allowing the biosynthesis of sialic acid its activation, its transport into the Golgi, and finally its transfer onto terminal galactose and mAbs coexpressed with engineered human sialylation pathway carried up to 80% sialylated structures [30]. Six mammalian enzymes were overexpressed in plants [34].

5 Applications

Advancement has been made in the design and development of plant expression systems for the generation of recombinant *N*-glycans glycoproteins by glycoengineering. *N*-glycosylation affects many properties of recombinant glycoproteins produced *in planta* including efficient plant-made antibodies for passive immunization but with shorter half-life in the blood due to a higher clearance rate [35–38]. The removal of the core fucose residue from mammalian α-1,6-fucose or the plant α-1,3-fucose from the *N*-glycan of an antibody has been reported to increase the antibody-dependent cellular cytotoxicity (ADCC) [1, 36, 37] thus proving as an effective biopharmaceutical. The Food and Drug Administration (FDA) has approved first plant-made pharmaceutical protein for human parenteral administration including taliglucerase alfa [38], also named as Elelyso, produced by Protalix Biotherapeutics for the application as a replacement therapy for Gaucher disease, which is advantageous due to the structure of the exposed terminal mannose residues on α-1,3-fucose- and β-1,2-xylose-containing *N*-glycan structures generated in plant cell vacuoles [1] that are required for the efficient uptake of the enzyme into macrophages. *N. benthamiana* has been extensively researched for the production of mucin-type *O*-glycans [39] and *N*-glycans [40] of recombinant proteins.

As plant cells lack β1,4-galactosylated and sialylated glycan, which have important biological functions in animal cells [1, 26], transgenic human β1,4-galactosyltransferase producing tobacco BY2 suspension-cultured cells were developed [1, 27]. Two genes encoding human CMP-*N*-acetylneuraminic acid synthetase and CMP-sialic acid transporter expressed in tobacco suspension-cultured cell to enable sialic acid biosynthesis in plants can act as bioreactor for mammalian glycoprotein production.

Human butyrylcholinesterase (BChE) is a tetrameric human serum sialylated protein that finds therapeutic importance as a candidate bioscavenger of organophosphorus nerve agents. *N. benthamiana* has been engineered for the expression of sialylated protein by transient co-expression of BChE cDNA by vectors [41] leading to the generation of rBChE expressing mono- and di-sialylated *N*-glycans in the intracellular fluid with similarity to the human protein orthologue. β1,4-*N*-acetylglucosaminyl-transferase IV overexpression in the recombinant engineered plant enabled the generation of branched N-glycans, with tri-sialylated structures with better and effective novel therapeutic role [42].

Glycoprotein hormone erythropoietin (EPO) finds importance in the maintenance of hematopoiesis and providing tissue protection and recombinant human EPO (rhuEPO) find application in the treatment of anemia. However, rhyEPO at higher doses can cause harmful increase in the RBC masses and reveals limited role in tissue protection. Asialoerythropoietin (asialo-rhuEPO), which is a desialylated form of rhuEPO, has been reported to lack hematopoietic activity, but retain cytoprotective activity. But chemically enzymatic desialylation of rhuEPO suffers from not being cost effective. Although plants are known to synthesize complex *N*-glycans, they lack enzymes to transfer sialic acid and β1,4-galactose to *N*-glycan chains, therefore serve as a potential expression for generation of asialoerythropoietin.

Asialo-rhuEPO is being designed to be produced in plants by introducing human β1,4-galactosyltransferase as the penultimate β1,4-linked galactose residues regulating its in vivo biological activity. Co-expression of human β1,4-galactosyltransferase and EPO genes in tobacco plants has been reported to accumulate asialo-rhuEPO confirmed by its specificity to *Erythrina cristagalli* lectin column, revealing expression of *N*-glycan structures with terminal β1,4-galactose residues and a functional co-expressed GalT. Asialo-rhuEPO has been reported to interact with the EPO receptor (EPOR) with similar affinity as rhuEPO with desired biological function [43].

N-glycans with terminal Neu5Ac residues are important for the biological activities and half-lives of recombinant therapeutic glycoproteins in humans but the fact that plants express negligible amounts of free or protein-bound Neu5Ac presents a major disadvantage for their application as biopharmaceutical expression system. Thus to synthesize Neu5Ac-containing *N*-glycans, plants need to synthesize Neu5Ac and its nucleotide-activated derivative, cytidine monophospho-*N*-acetylneuraminic acid. Transgenic *A. thaliana* plants expressing three key enzymes of the mammalian Neu5Ac biosynthesis pathway, UDP-*N*-acetylglucosamine

2-epimerase/*N*-acetylmannosamine kinase, *N*-acetylneuraminic acid phosphate synthase, and CMP-*N*-acetylneuraminic acid synthetase, has been designed and developed and their simultaneous expression has led to the generation of significant Neu5Ac amounts in planta, which could be further converted to cytidine monophospho-*N*-acetylneuraminic acid by the coexpression of CMP-*N*-acetylneuraminic acid synthetase leading to the production of Neu5Ac-containing glycoproteins in plants [44].

Neu5Ac could be synthesized in the plant cytosol by the expression of microbial Neu5Ac-synthesizing enzymes including Neu5Ac lyase from *Escherichia coli* and Neu5Ac synthase (neuB2) from *Campylobacter jejuni* in two model plants including Bright Yellow 2 (BY2) tobacco cells and *Medicago sativa* [45].

Human CMP-*N*-acetylneuraminic acid (NeuAc) synthase (HCSS) and α2,6-sialyltransferase (HST) enable sialylation of N-linked glycans in mammalian cells. HCSS synthesizes CMP-NeuAc, which HST uses as a donor substrate to transfer NeuAc to the terminal position of *N*-linked glycans. HCSS and HST genes could be inserted and expressed by the suspension-cultured tobacco BY2 cells to enable sialylation pathway in plants, producing mammalian-type sialoglycoproteins with terminal NeuAc residues in plants [46].

Two engineered constructs containing either the native signal peptide from human lactoferrin or the signal peptide from sweet potato sporamin fused to human lactoferrin has been reported to produce N-terminal sequences of rhLf purified from tobacco identical to Lf from human milk for both constructs [47].

The natural insertion of *N. benthamiana* into the RNA-dependent RNA polymerase 1 gene [1, 49] enables rapid production of high-value hormones, enzymes, and antibodies, and is successful in the production of ZMapp which is a cocktail of neutralizing mAb c13C6 and two chimeric antibodies c2G4 and c4G7, which were applied during the 2014–15 Ebola outbreak [48], and for the efficient production of vaccines against seasonal flu [49].

The intravenous immunoglobulin therapeutic application of ZMapp involves direct reaction to the virus and bind as lock and key leading to its deactivation and provides simulated immune response against Ebolaviral proteins Ebolavirus. Genes of the Ebola antibodies needed for the drug are inserted into Agrobacterium, then tobacco plants are injected or infused with the engineered viral vector-encoding Ebola antibodies, and plants produce the antibodies which are later isolated to form the drug known as ZMapp (Figs. 6 and 7, Table 2) [48].

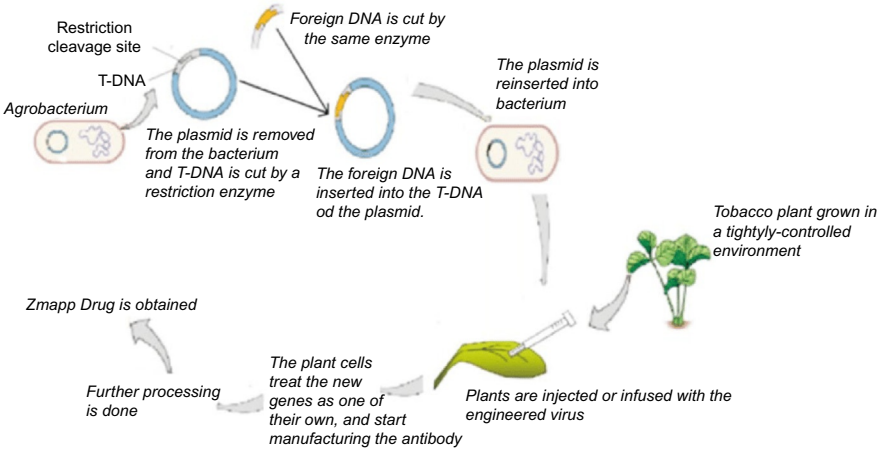

Fig. 6 Production of ZMapp through tobacco plant. *(Reproduced with permission from Zahara K, Bibi Y, Ajmal M, Sadaf HM, Bibi F, Sardar N, Riaz I, Laraib S. J Coast Life Med 2017;5:206–11.)*

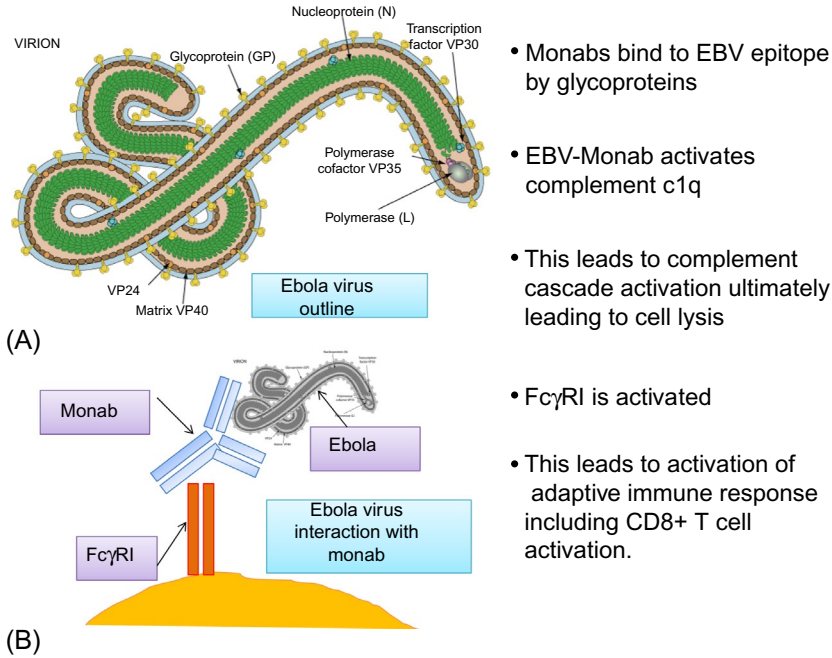

Fig. 7 (A) *Ebola* and (B) *Ebola* and interaction with ZMapp. *((A) Reproduced with permission Ebola picture Source ViralZone, SIB Swiss Institute of Bioinformatics.)*

Table 2 Plant produced human pharmaceuticals and industrial production

Company	Host	Lead product	Expression technology	Advantage	Website references
Mapp Biopharmaceutical/ LeafBiol, USA	Tobacco leaves	ZMapp for Ebola crisis	MagnICON Transient expression	Speed	[52]
Protalix, Carmiel, Israel	Carrot or tobacco cell culture	ELELYSO (taliglucerase alfa) Enzyme replacement	ProCellEx Stable Expression	Quality	[53]
Icon Genetics, München, Germany	*Nicotiana benthamiana* leaves	Vaccine for non-Hodgkin's Lymphoma	MagnICON Transient expression	Speed and Personalization	[54]
Ventria Bioscience, Junction City, KS, USA	Rice seeds	VEN150 for HIV-associated chronic inflammation	Express Tec Stable Expression	Scale Cost	[55]
Greenovation Biotech GmbH, Heilbronn, Germany	Moss	Moss-GAA for Pompe Disease, Moss-GBA for Gaucher's Disease, Moss-AGAL for Fabry Disease	Moss *Physcomitrella patens*-based Broytechnolgy	Speed Scale and Customized	[56]
Kentucky BioProcessing, Owensboro, KY, USA	*Nicotiana benthamiana* leaves	Contract service	Geneware Transient expression	Speed	[57]
PhycoBiologics Inc. Bloomington, IN, USA	Algae	Vaccines Growth Factor and enzymes	Microalgae expression	Speed Scale	[58]
Medicago, Québec, QC, Canada	*Nicotiana benthamiana* Alfalfa	Vaccine for influenza, Pandemic market, Rabies and Rotavirus	Proficia Transient Expression; Stable Expression	Speed	[59]

Continued

Table 2 Plant produced human pharmaceuticals and industrial production—cont'd

Company	Host	Lead product	Expression technology	Advantage	Website references
Synthon, Nijmegen, The Netherlands	Duckweed LeafyBiomass	Antibody for non-Hodgkin's Lymphoma	LEX system Stable expression	Speed Quality	[60]
Fraunhofer IME, Aachen, Germany	Tobacco leaves	HIV Antibody	Stable Nuclear Expression	Scale Cost	[61]
Fraunhofer CMB/iBio, Newark, DE, USA	*Nicotiana benthamiana* leaves	Influenza vaccine	Transient expression	Speed	[62]
Healthgen, Wuhan, Hubei, China	Rice seed	Serum albumin	Stable Expression	Quality Scale	[63]
PlanetBiotechnology, Hayward, CA, USA	Tobacco leaves	CaroRx for dental caries; PBI-220 antibody for anthrax; DPP4-Fc for MERS coronavirus infection	Stable Expression	Quality Scale	[64]

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6 Mucin type *O*-glycans and plant expression

As plants lack endogenous glycosyltransferase that lead to Ser/Thr glycosylation as in mammals, plants find importance for the engineering of *O*-glycosylation as there are no endogenous glycosyltransferases that can act upon engineered enzymes for the synthesis of *O*-glycans. Transient expression of mucin-type *O*-GalNAc and core 1 O-linked glycan structures on recombinant human erythropoietin fused to an IgG heavy chain fragment (EPO-Fc) have been reported to be synthesized in *N. benthamiana* plants. Sialylated core structure constructs encoding human polypeptide:*N*-acetylgalactosaminyltransferase, *Drosophila melanogaster* core 1 β1,3-galactosyltransferase, human α2,3-sialyltransferase, and *Mus musculus* α2,6-sialyltransferase have been reported for their transient co-expression in *N. benthamiana* together with EPO-Fc leading to the synthesis of mono- and disialylated *O*-linked glycans and biantennary structures with terminal sialic acid residues [65].

Engineering of *O*-linked glycans is not much developed in plant systems as the *O*-glycosylation pathways in plants are different from that in human. In mammals *O*-glycans on secretory proteins are formed by the attachment of *N*-acetylgalactosamine (GalNAc) to serine or threonine residues (mucin-type *O*-glycosylation) which are further modified by the addition of different monosaccharides such as galactose, GlcNAc, sialic acid, forming mucin-type core *O*-glycan structures that is important in different biological processes [66] (Fig. 8).

In plants, unlike mammals, proline residues are converted to hydroxyproline (Hyp) by prolyl-4-hydroxylases (P4H) that are linked with arabinose residues. Knockout of P4H genes could eliminate *O*-glycosylation in *P. patens*, *thereby helping in the* modification of recombinantly expressed EPO [1, 6, 67]. Overexpression of human polypeptide GalNAc-transferase 2 (GalNAcT2) in *Arabidopsis*, tobacco BY2 cells, and *N. benthamiana* [68–70], initiating *O*-GalNAc formation on different recombinant glycoproteins (including EPO and IgA1 antibodies) [71], has been reported. This GalNAc residue acts as a substrate for subsequent elongation with β1,3-galactose by overexpressing β1,3-galactosyltransferase (C1GalT1) and expression of C1GalT1 and genes for the human sialylation pathway enabled the synthesis of sialylated *O*-glycans [6, 72].

7 Introducing helminth glycosylation into plants

Parasitic helminths secrete immunomodulatory with certain *N*-glycan epitopes including Lewis X and LDN-F glycan motives that find importance in treatment of allergies and autoimmune diseases. Overexpression of glycosyltransferases including FucTs, GalTs, and GalNAcTs in *N. benthamiana*

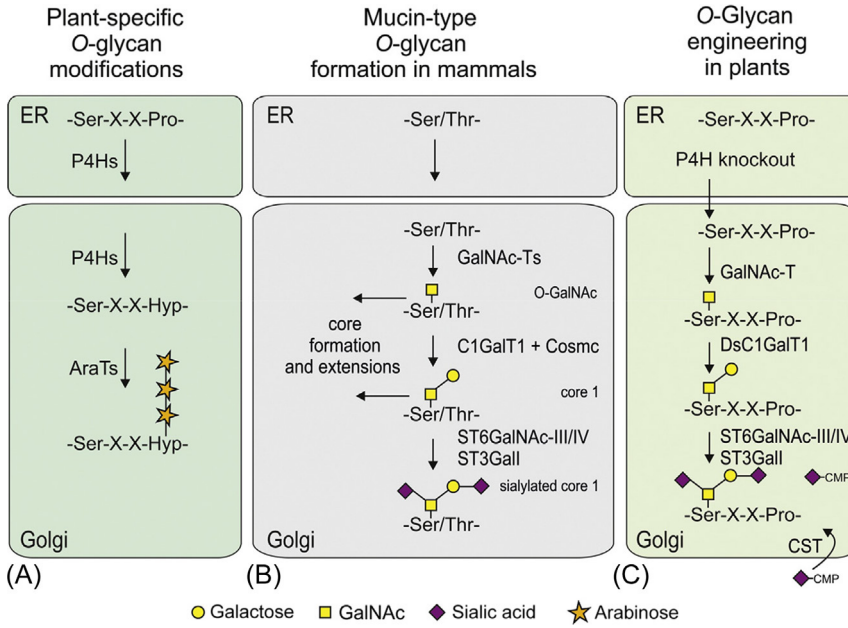

Fig. 8 (A) Schematic representation of plant-type O-glycosylation. Proline residues adjacent to *O*-glycosylation sites are converted to hydroxyproline (Hyp) by prolyl-4-hydroxylases (P4Hs). Hyp residues are further elongated (e.g., by arabinosyltransferases—AraTs). (B) Mucin-type *O*-glycan biosynthesis pathway in mammals. Polypeptide GalNAc-transferases (GalNAc-Ts), β1,3-galactosyltransferases 1 (C1GalT1), Cosmc (chaperone), sialyltransferases (ST6GalNAcIII/IV, ST3GalI). (C) Mucin-type *O*-glycan-engineering in plants. Strategies involve the knockout of P4Hs to prevent Hyp formation and expression of mammalian GalNAc-Ts, Drosophila melanogasterc1galt1, and STs. *(Reproduced with permission from Schoberer J Strasser R, Plant glyco-biotechnology.Semin Cell Dev Biol. 2018 80:133-141.)*

enabled the reconstruction of Lewis X and LDN-F motives [73] that find importance in the development of anti-helminthic vaccines.

8 Detection

Detection of low-level monosaccharides in the glycoprotein hydrolyzate are accomplished by derivatization prior to high-performance liquid chromatography (HPLC)-fluorescence and liquid chromatography (LC)-sonic spray ionization (SSI)-mass spectrometry (MS) analyses. LC-SSI-MS has been employed to identify the compositional monosaccharides including glucosamine, glucose, mannose, arabinose, xylose, and sialic acid found in the transgenic corn [74].

9 Discussion

In plant biomanufacturing of human proteins of importance, glycosylation, is one of the most addressed PTMs, as it affects protein homogeneity and functionality. Different engineering expression systems have been designed to control glycosylation and generate engineered *N*- and *O*-linked glycans with targeted sugar profiles and their various applications in the generation of human therapeutics [1–78].

Despite advances in the study of *N*-glycosylation pathways in plants, the study is far from complete and not completely known as compared to the human *N*-glycosylation pathway. The *N*-glycosylation pathway is not completely known for model plant organism *A. thaliana* and other different plant species. Although intra-Golgi glycosyltransferases are reported in *A. thaliana*, their functions remain unknown [79] but is assumed to play a vital role in synthesis of *O*-glycosylated proteins like arabinogalactan proteins. Studies from *A. thaliana* and rice have indicated that *N*-glycans enable growth under stress. However, complete genome sequencing of different plants will enable better understanding of the N-glycan pathway in plants and their efficient modifications and research in this exciting field of biology with human applications in the generation of therapeutics compatible to the human body is increasing across the globe.

References

[1] Loos A, Steinkellner H. IgG-fc glycoengineering in non-mammalian expression hosts. Arch Biochem Biophys 2012;526:167–73.
[2] Sack M, Hofbauer A, Fischer R, Stoger E. The increasing value of plant-made proteins. Curr Opin Biotechnol 2015;32:163–70.
[3] Bally J, Nakasugi K, Jia F, Jung H, Ho SY, Wong M, et al. The extremophile *Nicotiana benthamiana* has traded viral defence for early vigour. Nat Plants 2015;1:15165.
[4] Montero-Morales L, Steinkellner H. Advanced plant-based glycan engineering. Front Bioeng Biotechnol 2018;6:81.
[5] Source Wikipedia: Joachim Müllerchen—Own work Multi-license with GFDL and Creative Commons CC-BY 2.5 https://commons.wikimedia.org/wiki/File:Tabak_9290019.JPG
[6] Yao J, Weng Y, Dickey A, Wang KY. Plants as factories for human pharmaceuticals: applications and challenges. Int J Mol Sci 2015;16(12):28549–65.
[7] Stührwohldt N, Schaller A. Regulation of plant peptide hormones and growth factors by post-translational modification. Plant Biol J 2018;.
[8] Nagashima Y, Schaewen A, Koiwa H. Function of N-glycosylation in plants review article. Plant Sci 2018;274:70–9.
[9] Schoberer J, Strasser R. Plant glyco-biotechnology. Semin Cell Dev Biol 2018;80:133–41.
[10] Daskalova SM, Pah AR, Baluch DP, Lopez LC. The Arabidopsis thaliana putative sialyltransferase resides in the Golgi apparatus but lacks the ability to transfer sialic acid. Plant Biol (Stuttg) 2009;11:284–99.

[11] Strasser R, Altmann F, Mach L, Glössl J, Steinkellner H. Generation of Arabidopsis thaliana plants with complex N-glycans lacking β1,2-linked xylose and core α1,3-linked fucose. FEBS Lett 2004;561:132–6.
[12] Cox KM, Sterling JD, Regan JT, Gasdaska JR, Frantz KK, Peele CG, Black A, et al. Glycan optimization of a human monoclonal antibody in the aquatic plant *Lemna minor*. Nat Biotechnol 2006;2006(24):1591–7.
[13] Sourrouille C, Marquet-Blouin E, D'Aoust M-A, Kiefer-Meyer M-C, Seveno M, Pagny-Salehabadi S, Bardor M, et al. Down-regulated expression of plant-specific glycoepitopes in alfalfa. Plant Biotechnol J 2008;6:702–21.
[14] Strasser R, Stadlmann J, Schähs M, Stiegler G, Quendler H, Mach L, Glössl J, et al. Generation of glyco-engineered *Nicotiana benthamiana* for the production of monoclonal antibodies with a homogeneous human-like N-glycan structure. Plant Biotechnol J 2008;6:392–402.
[15] Weterings, K. and Van Eldik, G. Nicotiana benthamiana plants deficient in fucosyltransferase activity. International patent application WO 2013/050155.
[16] Hanania U, Ariel T, Tekoah Y, Fux L, Sheva M, Gubbay Y, Weiss M, et al. Establishment of a tobacco BY2 cell line devoid of plant specific xylose and fucose as a platform for the production of biotherapeutic proteins. Plant Biotechnol J 2017;15:1120–9.
[17] Li J, Stoddard TJ, Demorest ZL, Lavoie PO, Luo S, Clasen BM, Cedrone F, et al. Multiplexed, targeted gene editing in Nicotiana benthamiana for glyco-engineering and monoclonal antibody production. Plant Biotechnol J 2016;14:533–42.
[18] Mercx S, Smargiasso N, Chaumont F, De Pauw E, Boutry M, Navarre C. Inactivation of the β(1,2)-xylosyltransferase and the α(1,3)-fucosyltransferase genes in Nicotiana tabacum BY-2 cells by a multiplex CRISPR/Cas9 strategy results in glycoproteins without plant-specific glycans. Front Plant Sci 2017;8:403.
[19] Zischewski J, Sack M, Fischer R. Overcoming low yields of plant-made antibodies by a protein engineering approach. Biotechnol J 2015;11:107–16.
[20] Gleba Y, Klimyuk V, Marillonnet S. Magnifection—a new platform for expressing recombinant vaccines in plants. Vaccine 2005;23:2042–8.
[21] Sainsbury F, Thuenemann EC, Lomonossoff GP. pEAQ:Versatile expression vectors for easy and quick transient expression of heterologous proteins in plants. Plant Biotechnol J 2009;7:682–93.
[22] Floss DM, Sack M, Stadlmann J, Rademacher T, Scheller J, Stöger E, Fischer R, et al. Biochemical and functional characterization of anti-HIV antibody–ELP fusion proteins from transgenic plants. Plant Biotechnol J 2008;6:379–91.
[23] Kim YG, Cha J, Chandrasegaran S. Hybrid restriction enzymes: Zinc finger fusions to Fok I cleavage domain. Proc Natl Acad Sci 1996;93:1156–60.
[24] Christian M, Cermak T, Doyle EL, Schmidt C, Zhang F, Hummel A, Bogdanove AJ, et al. Targeting DNA double-strand breaks with TAL effector nucleases. Genetics 2010;186:757–61.
[25] Jansing J, Sack M, Augustine SM, Fischer R, Bortesi L. CRISPR/Cas9-mediated knockout of six glycosyltransferase genes in Nicotiana benthamiana for the production of recombinant proteins lacking β-1,2-xylose and core α-1,3-fucose. Plant Biotechnol J 2019;17:350–61.
[26] Kallolimath S, Castilho A, Strasser R, Grünwald-Gruber C, Altmann F, Strubl S, Galuska CE, Zlatina K, Galuska SP, Werner S, Thiesler H, Werneburg S, Hildebrandt H, Gerardy-Schahn R, Steinkellner H. Engineering of complex protein sialylation in plants. Proc Natl Acad Sci U S A 2016;113:9498–503.
[27] Palacpac NQ, Yoshida S, Sakai H, Kimura Y, Fujiyama K, Yoshida T, et al. Stable expression of human beta1,4-galactosyltransferase in plant cells modifies *N*-linked glycosylation patterns. Proc Natl Acad Sci U S A 1999;96:4692–7.
[28] Bakker H, Rouwendal GJ, Karnoup AS, Florack DE, Stoopen GM, Helsper JP, et al. An antibody produced in tobacco expressing a hybrid beta-1,4-galactosyltransferase is essentially devoid of plant carbohydrate epitopes. Proc Natl Acad Sci U S A 2006;103:7577–82.

[29] Castilho A, Strasser R, Stadlmann J, Grass J, Jez J, Gattinger P, Kunert R, Quendler H, Pabst M, Leonard R, Altmann F, Steinkellner H. In planta protein sialylation through overexpression of the respective mammalian pathway. J Biol Chem 2010;285(21):15923–30.
[30] Ko K, Tekoah Y, Rudd PM, Harvey DJ, Dwek RA, Spitsin S, Hanlon CA, et al. Function and glycosylation of plant-derived antiviral monoclonal antibody. Proc Natl Acad Sci 2003;100:8013–8.
[31] Forthal DN, Gach JS, Landucci G, Jez J, Strasser R, Kunert R, Steinkellner H. Fc-glycosylation influences Fcγ receptor binding and cell-mediated anti-HIV activity of monoclonal antibody 2G12. J Immunol 2010;185:6876–68824.
[32] Strasser R, Castilho A, Stadlmann J, Kunert R, Quendler H, Gattinger P, Jez J, Rademacher T, Altmann F, Mach L, Steinkellner H. J Biol Chem 2009;284:20479–85.
[33] Castilho A, Bohorova N, Grass J, Bohorov O, Zeitlin L, Whaley K, Altmann F, Steinkellner H. Plos One 2011;6.
[34] Loos A, Steinkellner H. Plant glyco-biotechnology on the way to synthetic biology. Front Plant Sci 2014;5:523.
[35] Castilho A, Strasser R, Stadlmann J, Grass J, Jez J, Gattinger P, Kunert R, Quendler H, Pabst M, Leonard R, Altmann F, Steinkellner H. In planta protein sialylation through overexpression of the respective mammalian pathway. J Biol Chem 2010;285:15923–30.
[36] Shields RL, Lai J, Keck R, O'Connell LY, Hong K, Meng YG, Weikert SHA, et al. Lack of fucose on human IgG1 N-linked oligosaccharide improves binding to human FcγRIII and antibody-dependent cellular toxicity. J Biol Chem 2002;277:26733–40.
[37] Zeitlin L, Pettitt J, Scully C, Bohorova N, Kim D, Pauly M, Hiatt A, et al. Enhanced potency of a fucose-free monoclonal antibody being developed as an Ebola virus immunoprotectant. Proc Natl Acad Sci 2011;108:20690–4.
[38] Tekoah Y, Tzaban S, Kizhner T, Hainrichson M, Gantman A, Golembo M, Aviezer D, et al. Glycosylation and functionality of recombinant β-glucocerebrosidase from various production systems. Biosci Rep 2013;33:e00071.
[39] Strasser R. Engineering of human-type O-glycosylation in Nicotiana benthamiana plants. Bioengineered 2013;4:191–6.
[40] Castilho A, Pia G, Grass J, Jez J, Pabst M, Altmann F, Gorfer M, Strasser R, Steinkellner H. N-Glycosylation engineering of plants for the biosynthesis of glycoproteins with bisected and branched complex *N*-glycans. Glycobiology 2011;21:813–23.
[41] Schneider JD, Castilho A, Neumann L, Altmann F, Loos A, Kannan L, Mor TS, Steinkellner H. Expression of human butyrylcholinesterase with an engineeredg-lycosylation profile resembling the plasma-derived orthologue. Plant Cell Rep 2012;31:1233–43.
[42] Schneider JD, Marillonnet S, Castilho A, Gruber C, Werner S, Mach L, Klimyuk V, Mor TS, Steinkellner H. Oligomerization status influences subcellular deposition and glycosylation of recombinant butyrylcholinesterase in Nicotiana benthamiana. Plant Biotechnol J 2014;12:832–9.
[43] Kittur FS, Hung CY, Darlington DE, Sane DC, Xie J. N-Glycosylation engineering of tobacco plants to produce asialoerythropoietin. Plant Cell Rep 2012;31:1233–43.
[44] Misaki R, Fujiyama K, Seki T. Expression of human CMP-N-acetylneuraminic acid synthetase and CMP-sialic acid transporter in tobacco suspension-cultured cell. Biochem Biophys Res Commun 2006;339:1184–9.
[45] Kajiura H, Misaki R, Fujiyama K, Seki T. Stable coexpression of two human sialylation enzymes in plantsuspension-cultured tobacco cells. J Biosci Bioeng 2011;111:471–7.
[46] Navarre C, Smargiasso N, Duvivier L, Nader J, Far J, De Pauw E, Boutry M. N-glycosylation of an IgG antibody secreted by Nicotiana tabacum BY-2 cells can be modulatedthrough co-expression of human β-1,4-galactosyltransferase. Transgenic Res 2017;26:375–84.

[47] Salmon V, Legrand D, Slomianny MC, el Yazidi I, Spik G, Gruber V, Bournat P, Olagnier B, Mison D, Theisen M, Mérot B. Production of human lactoferrin in transgenic tobacco plants. Protein Expr Purif 1998;13:127–35.
[48] Qiu X, Wong G, Audet J, Bello A, Fernando L, Alimonti JB, et al. Reversion of advanced Ebola virus disease in nonhuman primates with ZMapp. Nature 2014;514:47–53.
[49] D'Aoust MA, Couture MM, Charland N, Trépanier S, Landry N, Ors F, et al. The production of hemagglutinin-based virus-like particles in plants: a rapid, efficient and safe response to pandemic influenza. Plant Biotechnol J 2010;8:607–19.
[50] Zahara K, Bibi Y, Ajmal M, Sadaf HM, Bibi F, Sardar N, Riaz I. Laraib S. J Coast Life Med 2017;5:206–11.
[51] Ebola picture Source ViralZone, SIB Swiss Institute of Bioinformatics.
[52] Mapp Biopharmaceutical, Inc. Home Page. Available online: http://mappbio.com.
[53] Protealix Biotherapeutics. Home Page. Available online: https://www.protalix.com.
[54] Icon Genetics GmbH. Home Page. Available online: https://www.icongenetics.com.
[55] Ventria Biosciences. Home Page. Available online: https://www.ventria.com.
[56] Greenovation Biotech GmbH. Home Page. Available online: https://www.greenovation.com.
[57] Kentucky BioProcessing Home Page. Available online: https://www.kbpllc.com.
[58] PhycoBiologics Inc., Home Page. Available online: https://www.phycotransgenics.com.
[59] Medicago. Home Page. Available online: https://www.medicago.com.
[60] Synthon. Home Page. Available online: http://www.synthon.com.
[61] Fraunhofer IME Home Page. Available online: http://www.ime.fraunhofer.de.
[62] Fraunhofer CMB. Home Page. Available online: http://www.fhcmb.org.
[63] Healthgen Biotechnology. Home Page. Available online: http://www.oryzogen.com.
[64] Planet Biotechnology Inc. Home Page. Available online: http://www.planetbiotechnology.com.
[65] Castilho A, Neumann L, Daskalova S, Mason HS, Steinkellner H, Altmann F, Strasser R. Engineering of sialylated mucin-type O-glycosylation in plants. J Biol Chem 2012 Oct 19;287(43):36518–26.
[66] Schjoldager KT, Clausen H. Site-specific protein O-glycosylation modulates proprotein processing—deciphering specific functions of the large polypeptide GalNAc-transferase gene family. Biochim Biophys Acta 1820;2012:2079–94.
[67] Parsons J, Altmann F, Graf M, Stadlmann J, Reski R, Decker EL. A gene responsible for prolyl-hydroxylation of moss-produced recombinant human erythropoietin. Sci Rep 2013;3:3019.
[68] Daskalova SM, Radder JE, Cichacz ZA, Olsen SH, Tsaprailis G, Mason H, et al. Engineering of *N. benthamiana* L. plants for production of N-acetyl-galactosamine-glycosylated proteins-towards development of a plant-based platform for production of protein therapeutics with mucin type O-glycosylation. BMC Biotechnol 2010;. 6750-10-62.
[69] Castilho A, Windwarder M, Gattinger P, Mach L, Strasser R, Altmann F, et al. Proteolytic and N-glycan processing of human alpha1-antitrypsin expressed in *Nicotiana benthamiana*. Plant Physiol 2014;166:1839–51.
[70] Yang WH, Aziz PV, Heithoff DM, Mahan MJ, Smith JW, Marth JD. An intrinsic mechanism of secreted protein aging and turnover. Proc Natl Acad Sci U S A 2015;112:13657–62.
[71] Dicker M, Maresch D, Strasser R. Glyco-engineering for the production of recombinant IgA1 with distinct mucin-type O-glycans in plants. Bioengineered 2016;2016(7):484–9.
[72] Castilho A, Neumann L, Daskalova S, Mason HS, Steinkellner H, Altmann F, et al. Engineering of sialylated mucin-type O-glycosylation in plants. J Biol Chem 2012;287:36518–26.

[73] Wilbers RH, Westerhof LB, van Noort K, Obieglo K, Driessen NN, Everts B, Gringhuis SI, Schramm G, Goverse A, Smant G, Bakker J, Smits HH, Yazdanbakhsh M, Schots A, Hokke CH. Production and glyco-engineering of immunomodulatory helminth glycoproteins in plants. Sci Rep 2017;7:45910.
[74] Wang T, Nam PK, Shi H, Ma Y. Compositional monosaccharide analysis of transgenic corn glycoproteins by HPLC with fluorescence detection and LC-MS with sonic spray ionization. J Chromatogr Sci 2007;45:200–6.
[75] Castilho A, Pabst M, Leonard R, Veit C, Altmann F, Mach L, Glössl J, Strasser R, Steinkellner H. Construction of a functional CMP-sialic acid biosynthesis pathway in Arabidopsis. Plant Physiol 2008;147:331–9.
[76] Chen M, Liu X, Wang Z, Song J, Qi Q, Wang PG. Modification of plant N-glycans processing: the future of producing therapeutic protein by transgenic plants. Med Res Rev 2005;25:343–60.
[77] Paccalet T, Bardor M, Rihouey C, Delmas F, Chevalier C, D'Aoust MA, Faye L, Vézina L, Gomord V, Lerouge P. Engineering of a sialic acid synthesis pathway in transgenic plants by expression of bacterial Neu5Ac-synthesizing enzymes. Plant Biotechnol J 2007;5:16–25.
[78] Nikolovski N, Rubtsov D, Segura MP, Miles GP, Stevens TJ, Dunkley TP, Munro S, Lilley KS, Dupree P. Putative glycosyltransferases and other plant Golgi apparatus proteins are revealed by LOPIT proteomics. Plant Physiol 2012;2012(160):1037–51.
[79] Parsons HT, Christiansen K, Knierim B, Carroll A, Ito J, Batth TS, Smith-Moritz AM, Morrison S, McInerney P, Hadi MZ, et al. Isolation and proteomic characterization of the Arabidopsis Golgi defines functional and novel components involved in plant cell wall biosynthesis. Plant Physiol 2012;2012(159):12–26.

Further reading

[80] Strasser R. Plant protein glycosylation. Glycobiology 2016;26:926–39.