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MINI REVIEW

Medicine Science International Medical Journal

Medicine Science 2022;11(1):447-51

Flexible approaches are required for successful production of recombinant proteins in plants

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Received 31 July 2021; Accepted 14 August 2021
Available online 20.01.2022 with doi: 10.5455/medscience.2021.07.242

Abstract

Production of recombinant proteins has increased significantly due to the ever-increasing demand in research laboratories, medical fields, and agriculture. Despite a variety of recombinant protein expression systems and gene engineering procedures exist owever, there is a limitation in achieving sufficient quantities of active, properly folded recombinant proteins. The selection of an appropriate expression system for the production of recombinant proteins should generally be chosen based on the biochemical and biological properties of the desired proteins to be produced. Plant expression platforms offer commercially inexpensive, safe, and fast scale-up properties for the production of recombinant proteins compared to traditional mammalian cell expression systems. However, the absence of some mammalian posttranslational modifications (PTMs) and aberrant N-glycosylation limit the use of these production systems. Therefore, molecular engineering of plant secretory pathways has provided an opportunity to develop next-generation pharmaceuticals with targeted biological functions. In this mini-review, we discuss the approaches that are needed for the production of functional recombinant proteins such as vaccines, antibodies, enzymes for medical, agricultural, and industrial applications.

Keywords: Glycosylation, N-glycans, plant-based expression systems, post-translational modifications, recombinant proteins

Introduction

Plant-based production system is an alternative expression platform that is potentially safe, scalable and reduces the production cost of targeted recombinant proteins compared to mammalian cell expression systems [1]. The plant-based expression systems have eukaryotic post-translational modifications machinery that affects the biological properties of the proteins. As is known, the majority of proteins undergo post-translational modifications, which is most important for functional activity, immunogenicity, protein-protein interaction, transport, stability, and also solubility of proteins [2]. In addition, if target proteins, such as malaria antigens, for example, Pfs48/45, Pfs25 and Pfs230 proteins of Plasmodium falciparum, also antigens of a wide range of bacteria do not carry N-linked glycans in their native glycan form, these proteins can be abnormally glycosylated in any eukaryotic system, thus, impairing the native folding and biological activity [3-6].

Recent observations demonstrated that low-yield and improper folding of proteins may be associated with specific processes, (such as glycosylation) in the plant secretory pathway [7]. In this mini-review, we evaluate the importance of flexible approaches and potential engineering pathways, which are important to improve the production of complex recombinant pharmaceuticals.

Approaches For The Production Of Functionally Active Recombinant Proteins In Plants

N-glycan Processing in Endoplasmic Reticulum (ER) of the plants

Despite the many advantages of using plant-based expression systems, there are some limitations such as specific proteases that lead to degradation of functionally active proteins [8]. Strategies such as targeting recombinant proteins to organelles, engineering the protein sequence to remove sensitive regions and co-expression of various protease inhibitors (PIs) can protect recombinant proteins from degradation [9].

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Retention of complex proteins with multiple disulfide bridges, to the ER (endoplasmic reticulum), would be more appropriate for synthesizing correctly folded proteins. The storage of therapeutic proteins in the ER prevents immunogenic glycans from being added to plant-made pharmaceuticals (PMPs) [10]. Retention of the proteins in ER lumen is based on the addition of H / KDEL sequences to the C-terminal end of the protein [11]. ER-resident proteins contain high 6-9 mannose type N-glycans (Man6-Man9) that are structurally same in plants, yeast and mammals [12, 13], but complex N-type glycans contain different structural features in plants and mammals [14]. However, non-immunogenic high-mannose-type N-glycans have a brief *in-vivo* half-life, therefore, the use of these glycans in passive immunotherapy can be turned into an advantage [15, 16]. Mannose-6-phosphate (M6P) modifications, which are very important for the storage of lysosomal proteins are absent in plants. In this line, the M6P elaboration pathway has been engineered in plant [17] that enabld the production of safe and affordable recombinant mucopolysaccharidosis for enzyme replacement therapy [18, 19].

Approaches for Humanizing Plant N-glycosylation Pathways

Therapeutic proteins generally need at least proteolytic cleavage and glycosylation for their bioactivity, stability, and solubility, [20]. As plants become a viable alternative system for animal and microbial cell factories for the production of therapeutic proteins, they require rearrangements to obtain glycans that are not immunogenic for human health [12]. Many plant-made pharmaceuticals (PMPs) such as antibodies, vaccine antigens, and hormones are glycoproteins. Glycosylation is the attachment of glycans to the peptide backbone of the protein by covalent bonds. N-linked glycosylation can affect the proper folding, stability, resistance to proteolysis, transport of proteins [21, 22].

The attachment of α1, 3-fucose and β1, 2-xylose to N-glycan core (GnGn) is one of the most important N-glycan formations in plants. These structures typical plant glycoform and present almost all analyzed plants [23]. The human glycoproteins do not carry these two residues and they can be very immunogenic for human health. So, the initial step in glycoengineering approaches in plants will be to eliminate these residues from glycans. Gene silencing (sense and antisense RNAi), genome editing, and knockout strategies were successfully applied to species *Nicotiana benthamiana (N. benthamiana)* [24], *Nicotiana tabacum (N. tabacum)* BY2 cells [25], *Physcomitrella patens (P. patens)* [26], *Lemna minor (L. minor)* [27] which are often used for the production of recombinant proteins. Additionally, recently, the plant-specific α1, 3-fucose, and β1, 2-xylose glycan residues have been completely removed with the CRISPR-Cas9-engineered *N. benthamiana* plant [28]. Also, it was demonstrated that α-fucosylated content rituximab and trastuzumab antibodies displayed importantly increased antibody-dependent-cell-cytotoxicity (ADCC) *in-vivo* contrasted to their fucosylated equivalents [29-31].

The formation of truncated N-glycans (paucimannosidic) produced by removing terminal GlcNAc (MMXF) residues from the core N-glycan is another limitation in plant-based expression systems. This structure is formed by the terminal cleavage of GlcNAc residues by hydrolases, b-N-acetylhexosaminidases (HEXO) [32]. Vacuolar glycoproteins and some extracellular plant glycoproteins contain Paucimannosidic N-glycans [14, 33]. In some cases, these truncated structures are useful for certain therapeutic applications, such as the use of recombinant human glucocerebrosidase produced by the carrot cell used in enzyme replacement therapies [18]. Also in most therapeutic applications, these structures are negatively impacting proteins functions [34]. Gene silencing and knockout strategies can eliminate hexosaminidase enzyme expressions, prevent GlcNAc hydrolysis from GnGn structures and and increase the expenditure of complex GlcNAc-terminator N-glycan of paucimannosidic structures [35].

In addition, elongation of the outer chain of N-glycans and Lewis A structures [23] extending from α 1,3-galactose (Gal) and α 1,4-fucose (Fuc) residues to the GlcNAc terminal [23, 36, 37] were detected in plants. Although Lewis A structures are found in human tissues, these structures negatively affect the activity and antigenicity of recombinant glycoproteins. Parsons et al. (2012), successfully demonstrated the production of erythropoietin lacking the Lewis A epitope by knockouts the α1,3-galactose and α1,4-fucose enzymes in Physcomitrella patens [38].

Another important strategy is GlycoDelete, which depends on the elimination of key N-acetyltgucosaminyltransferaseI (GanTI) enzyme activity, which converts Man5 structures into complex N-glycans. The GlycoDelete strategy shortens the N-glycosylation (Golgi) pathway in mammalian cells. Using this strategy, recombinant proteins are produced by small, sialylated trisaccharide N-glycans.[40]. This strategy can be advantageous for the production of monoclonal antibodies and growth factors [39].

In plant cells N-glycosylation site such as Asn-X-Cys [41] can be used which is another alternative of Oligosaccharyltransferase (OST) but it has not been described for endogenous plant proteins [42]. In the ER lumen, pre-assembled oligosaccharides transfers to asparagine residues of protein N-glycans which catalyzes Oligosaccharyltransferase (OST) [41].

Approaches for the Increasing Plant Glycosylation Capacities:- Genomic Insertions

Although the formation of GnGn structures in mammals and plants is the same, plants cannot provide additional variations in N-glycans. Bakker et al. (2001), reported that plant expressing human β-(1,4)-galactosyltransferase with a plant expressing the heavy and light chains of a mouse antibody results in the expression of a plantibody that exhibits partially galactosylated N-glycans (30%), which is approximately as abundant as when the same antibody is produced by hybridoma cell [43, 44].

The absence of sialic acid modifications limits the use of a plant transient expression system for the production of important mammalian derived pharmaceuticals and therapeutic enzymes. t was demonstrated that addition of negatively charged sialic acid groups on N-glycans can increase cell signaling, stability [45] and clearance rate of proteins [46]. Thus, it is very important to develop approaches to produce sialylated N-glycans in plants which result humanized N-glycan compositions of mammalian proteins and enzymes. To address this need, the production of sialic acid and human-type N-glycans was achieved by the expression of transient stable or suitable enzymes in the *N. benthamiana* plant [8, 47].

O-linked Glycosylation

The second major glycosylation type is O-linked glycosylation. Despite N-glycan engineering there is little attention was paid to plant O-linked glycan engineering. In mammalians, O-glycosylation is mucin-type, which O-glycans commonly attach N-acetylgalactosamine (GalNAc) to threonine or serine residues [48]. The knockout strategy was applied in *P. patens* to eliminate the specific O-glycans [49]. In *N. benthamiana* plants gene engineering is more challenging but, recent investigations in gene engineering allow eliminating O-glycans from the plant secretory pathway [50].

Another approach in plant-glycoengineering is the synthesis of Lewis-X epitopes (Lex) in *N. benthamiana* plants with the overexpression of specific glycosyltransferases [51]. Due to the stimulation of antigen-specific immune responses Lex-containing structures [52], the plant produced Lex epitopes may be found as a probable host for the production of vaccines [51].

In-vivo Deglycosylation Strategy

Plant expression system has many advantages over other expression systems. However, plant expression systems may not be suitable for proteins that do not carry N-linked glycans in their native glycan form. The *in-vivo* deglycosylation strategy of proteins with deglycosylation enzymes is considered an important strategy for the production of recombinant proteins by natural glycan formation in plants. [4, 5, 53]. The addition of glycan groups to the protein chain can strongly alter the biological behavior of proteins [54], can mask important epitopes of proteins, leading to incorrect folding of the proteins [4, 5]. Recently, Mamedov et al., (2012), developed an in vivo deglycosylation of plant-made recombinant proteins, by co-expressing with bacterial deglycosylation enzymes PNGaseF [4-6] and EndoH (Endo-β-N-acetylglucosaminidase) [53] using plant transient expression systems in N. benthamiana plants. The results have demonstrated that PNGaseF *in-vivo* deglycosylated proteins are highly immunogenic and appear to be more stable than their glycosylated counterparts [4-6]. It should be noted that PNGaseF removes N-glycans from glycoproteins, causing amino acid changes in the expressed proteins due to the deamidation reaction [53]. On the other hand, EndoH catalyze the bond between the two acetylglucosamine residues of the N-linked oligosaccharides while preserving the native protein structure without amino acid changes in the produced proteins [53]. Moreover, EndoH *in-vivo* deglycosylated proteins have superior properties compared to PNGaseF counterparts and glycosylated form of the same protein [54, 55]. For example, EndoH *in-vivo* deglycosylated form of Protective antigen (PA) of Bacillius anthracis was a more stable, active and highly immunogenic compared to its PNGaseF deglycosylated counterpart [54, 55].

Protein Processing

Most therapeutic proteins usually require proteolytic cleavage for their activation and other functions. Human furin is a very important enzyme for activating a wide range of proteins including cell surface receptor proteins, blood clotting factors, growth factors, hormones, and their receptors [56].

Tyrosine sulfation is of the mammalian cell PTMs and widely defines neutralizing antibodies targeting the HIV envelope glycoproteins [57]. There is only a report for tyrosine sulfation engineering in a plant-based expression system [58]. Additionally, γ-carboxylation is one of the important PTMs that affect the activity of coagulation factors. In theory, γ-carboxylase can be express in plant secretory pathways requiring additional PTMs. Mamedov et al., (2019a), demonstrated the transiently co-expression of blood-clotting factor IX was in N. benthamiana [54].

Conclusion

Plant expression systems are a promising platform for safe and cost-effective production of valuable recombinant proteins for medical, agricultural and industrial applications. However, plant expression system has limits for the expression of certain proteins. Recently, important progresses have been developed in the production of PMPs. The elimination of undesirable glycosylation enzymes improved the biological activity of therapeutic proteins like antibodies. Increasing expression of transferases enabled the production of desired complex N- and O-glycans. Also, the findings of the functions on polySia in neurological and immunological [59] settings, lead the new ways application of these strategies in medical areas. Additionally, new strategies like CRISPR/Cas9, leading to the development of plant and cell lines without fucose and xylose residues [60]. With the *in-vivo* deglycosylation strategy highly important vaccines candidates have been produced [4, 5, 54]. Taken all together the glyco-engineering strategies, -combined with plant-based expression systems provide the production of safe, functionally active and affordable pharmaceuticals. In this mini-review, we discussed possible important approaches for the successful production of valuable recombinant proteins in plant-based expression systems. Over the years, flexible approaches will continuously evolve as techniques for the production of recombinant proteins evolve.

Conflict of interests
The authors declare that they have no competing interests.

Financial Disclosure
All authors declare no financial support.

References

1. Nandi S, Kwong AT, Holtz BR, et al. Techno-economic analysis of a transient plant-based platform for monoclonal antibody production. mAbs. 2016;8:1456-66.
2. Gomord V, Faye L. Posttranslational modification of therapeutic proteins in plants. Curr Opin Plant Biol. 2004;7:171-81.
3. Chichester JA, Manceva SD, Rhee A, et al. A plant-produced protective antigen vaccine confers protection in rabbits against a lethal aerosolized challenge with Bacillus anthracis Ames spores. Hum Vaccin Immunother. 2013;9:544–52.
4. Mamedov T, Ghosh A, Jones RM, et al. Production of non-glycosylated recombinant proteins in Nicotiana benthamiana plants by co-expressing bacterial PNGase F. Plant Biotechnol J. 2012;10:773-82.
5. Mamedov T, Yusibov V. In vivo deglycosylation of recombinant proteins in plants by co-expression with bacterial PNGase F. Bioengineered. 2013;4:338-42.
6. Mamedov T, Chichester JA, Jones RM, et al. Production of functionally active and immunogenic non-glycosylated protective antigen from bacillus anthracis in nicotiana benthamiana by co-expression with peptide-n-glycosidase f (PNGase F) of flavobacterium meningosepticum. PLoS One. 2016;11:e0153956.
7. Fischer R, Buyel JF. Molecular farming - The slope of enlightenment.

Biotechnol Adv. 2020;40:107519.

8. Castilho A, Strasser R, Stadlmann J, et al. In planta protein sialylation through overexpression of the respective mammalian pathway. J Chem Biol. 2010;285:15923-930.

9. Grosse-Holz F, Madeira L, Zahid MA, et al. Three unrelated protease inhibitors enhance accumulation of pharmaceutical recombinant proteins in Nicotiana benthamiana. Plant Biotechnol J. 2018;16:1797–810.

10. Gomord V, Sourrouille C, Fitchette AC, et al. Production and glycosylation of plant-made pharmaceuticals: the antibodies as a challenge. Plant Biotechnol J. 2004;2:83-100.

11. Gomord V, Denmat LA, Fitchette-Lainé AC, et al. The C-terminal HDEL sequence is sufficient for retention of secretory proteins in the endoplasmic reticulum (ER) but promotes vacuolar targeting of proteins that escape the ER. Plant J. 1997;11:313-25.

12. Saint-Jore-Dupas C, Faye L, Gomord V. From planta to pharma with glycosylation in the toolbox. Trends Biotechnol. 2007;25:317-23.

13. Strasser R, Castilho A, Stadlmann J, et al. Improved virus neutralization by plant-produced anti-HIV antibodies with a homogeneous beta1,4-galactosylated N-glycan profile. J Biol Chem. 2009;284:20479–485.

14. Lerouge P, Cabanes-Macheteau M, Rayon C, et al. N-glycoprotein biosynthesis in plants: recent developments and future trends. Plant Mol Biol. 1998;38:31-48.

15. Pagny S, Cabanes-Macheteau M, Gillikin JW, et al. Protein recycling from the Golgi apparatus to the endoplasmic reticulum in plants and its minor contribution to calreticulin retention. Plant Cell. 2000;12:739-56.

16. Sriraman R, Bardor M, Sack M, et al. Recombinant anti-hCG antibodies retained in the endoplasmic reticulum of transformed plants lack core-xylose and core-alpha(1,3)-fucose residues. Plant Biotechnol J. 2004;2:279-87.

17. Zeng Y, He X, Danyukova T, et al. Toward Engineering the Mannose 6-Phosphate Elaboration Pathway in Plants for Enzyme Replacement Therapy of Lysosomal Storage Disorders. J Clin Med. 2019;812:2190.

18. Shaaltiel Y, Bartfeld D, Hashmueli S, et al. Production of glucocerebrosidase with terminal mannose glycans for enzyme replacement therapy of Gaucher's disease using a plant cell system. Plant Biotechnol J. 2007;5:579-90.

19. Van Patten SM, Hughes H, Huff MR, et al. Effect of mannose chain length on targeting of glucocerebrosidase for enzyme replacement therapy of Gaucher disease. Glycobiology. 2007;17:467-78.

20. Gomord V, Fitchette AC, Menu-Bouaouiche L, et al. Plant-specific glycosylation patterns in the context of therapeutic protein production. Plant Biotechnol J. 2010;8:564-87.

21. Helenius A, Aebi M. Intracellular functions of N-linked glycans. Science. 2001;291:2364-9.

22. Helenius A, Aebi M. Roles of N-linked glycans in the endoplasmic reticulum. Annu Rev Biochem. 2004;73:1019-49.

23. Wilson IB, Zeleny R, Kolarich D, et al. Analysis of Asn-linked glycans from vegetable foodstuffs: widespread occurrence of Lewis a, core alpha1,3-linked fucose and xylose substitutions. Glycobiology. 2001;11:261-74.

24. Li J, Stoddard TJ, Demorest ZL, et al. Multiplexed, targeted gene editing in Nicotiana benthamiana for glyco-engineering and monoclonal antibody production. Plant Biotechnol J. 2016;14:533-42.

25. Hanania U, Ariel T, Tekoah Y, Fux L, 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;15:1120-29.

26. Koprivova A, Stemmer C, Altmann F, Hoffmann A. Targeted knockouts of Physcomitrella lacking plant-specific immunogenic N-glycans. Plant Biotechnol J. 2004;2:517-23.

27. Cox KM, Sterling JD, Regan JT, Gasdaska JR, et al. Glycan optimization of a human monoclonal antibody in the aquatic plant Lemna minor. Nat. Biotechnol. 2006;24:1591–97.

28. 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. 2020;17:350-61.

29. Junttila TT, Parsons K, Olsson C, et al. Superior in vivo efficacy of afucosylated trastuzumab in the treatment of HER2-amplified breast cancer. Cancer Res. 2010;70:4481-9.

30. Gasdaska JR, Sherwood S, Regan JT, Dickey LF. An afucosylated anti-CD20 monoclonal antibody with greater antibody-dependent cellular cytotoxicity and B-cell depletion and lower complement-dependent cytotoxicity than rituximab. Mol Immunol. 2012;50:134-41.

31. Salles G, Morschhauser F, Lamy T, et al. Phase 1 study results of the type II glycoengineered humanized anti-CD20 monoclonal antibody obinutuzumab (GA101) in B-cell lymphoma patients. Blood.2012;119:5126-32.

32. Liebminger E, Veit C, Pabst M, et al. Beta-N-acetylhexosaminidases HEXO1 and HEXO3 are responsible for the formation of paucimannosidic N-glycans in Arabidopsis thaliana. J Biol Chem. 2011;286:10793-802.

33. Melo NS, Nimtz M, Conradt HS, et al. Identification of the human Lewis(a) carbohydrate motif in a secretory peroxidase from a plant cell suspension culture (Vaccinium myrtillus L.). FEBS Lett. 1997;41:186–91.

34. Yang WH, Aziz PV, Heithoff DM, et al. An intrinsic mechanism of secreted protein aging and turnover. Proc Natl Acad Sci U S A. 2015;112:13657-62.

35. Shin YJ, Castilho A, Dicker M, et al. Reduced paucimannosidic N-glycan formation by suppression of a specific β-hexosaminidase from Nicotiana benthamiana. Plant Biotechnol J. 2017;15:197-206.

36. Fitchette-Lainé AC, Gomord V, Cabanes M, et al. N-glycans harboring the Lewis a epitope are expressed at the surface of plant cells. Plant. 1997;12:1411-7.

37. Strasser R, Stadlmann J, Svoboda B, et al. Molecular basis of N-acetylglucosaminyltransferase I deficiency in Arabidopsis thaliana plants lacking complex N-glycans. Biochem J. 2005;387:385-91.

38. Parsons J, Altmann F, Arrenberg CK, et al. Moss-based production of asialo-erythropoietin devoid of Lewis A and other plant-typical carbohydrate determinants. Plant Biotechnol J. 2012;10:851-61.

39. Meuris L, Santens F, Elson G, Festjens N, et al. GlycoDelete engineering of mammalian cells simplifies N-glycosylation of recombinant proteins. Nat Biotechnol. 2004;32:485-9.

40. Piron R, Santens F, De Paepe A, et al. Using GlycoDelete to produce proteins lacking plant-specific N-glycan modification in seeds. Nat Biotechnol. 2015;33:1135-7.

41. Matsui T, Takita E, Sato T, et al. N-glycosylation at noncanonical Asn-X-Cys sequences in plant cells. Glycobiology. 2001;21:994-9.

42. Zielinska DF, Gnad F, Wiśniewski JR, Mann M. Precision mapping of an in vivo N-glycoproteome reveals rigid topological and sequence constraints. Cell. 2010;141:897-907.

43. Bakker H, Bardor M, Molthoff JW, Gomord V, et al. Galactoseextended glycans of antibodies produced by transgenic plants. Proc Natl Acad Sci U S A. 2001;98:2899-904.

44. Montero-Morales L, Steinkellner H. Advanced plant-based glycan engineering. Front Bioeng Biotechnol. 2018;6:81.

45. Kallolimath S, Castilho A, Strasser R, et al. Engineering of complex protein sialylation in plants. Proc Natl Acad Sci U S A. 2016;113:9498-503.

46. Sato C, Kitajima K. Disialic, oligosialic and polysialic acids: distribution, functions and related disease. J Biochem. 2013;154:115-36.

47. Castilho A, Neumann L, Daskalova S, et al. Engineering of sialylated mucin-type O-glycosylation in plants. J Biol Chem. 2012;287:36518-26.

48. 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. 2012;1820:2079-94.

49. Parsons J, Altmann F, Graf M, Stadlmann J. A gene responsible for prolyl-hydroxylation of moss-produced recombinant human erythropoietin. Sci Rep. 2016;22:3019.

50. Schiavinato M, Strasser R, Mach L, Dohm JC. Genome and transcriptome characterization of the glycoengineered Nicotiana benthamiana line ΔXT/FT. BMC Genom Data. 2019;20:594.

51. Rouwendal GJ, Wuhrer M, Florack DE, et al. Efficient introduction of a bisecting GlcNAc residue in tobacco N-glycans by expression of the gene encoding human N-acetylglucosaminyltransferase III. Glycobiology. 2007;17:334-44.

52. Wang J, Zhang Y, Wei J, et al. Lewis X oligosaccharides targeting to

DC-SIGN enhanced antigen-specific immune response. Immunology. 2007;121:174-82.

53. Mamedov T, Cicek K, Gulec B, et al. In vivo production of non-glycosylated recombinant proteins in Nicotiana benthamiana plants by co-expression with Endo-β-N-acetylglucosaminidase H (Endo H) of Streptomyces plicatus. PLoS One. 2017;12:e0183589.

54. Mamedov T, Musayeva I, Acsora R, et al. Engineering, and production of functionally active human Furin in N. benthamiana plant: In vivo post-translational processing of target proteins by Furin in plants. PLoS One. 2019a;14:e0213438.

55. Mamedov T, Cicek K, Miura K, et al. A Plant-Produced in vivo deglycosylated full-length Pfs48/45 as a Transmission-Blocking Vaccine Candidate against malaria. Sci Rep. 2019b;9:9868.

56. Nakayama K. Furin: a mammalian subtilisin/Kex2p-like endoprotease involved in processing of a wide variety of precursor proteins. Biochem J. 1997;327:625–35.

57. Lee JH, Andrabi R, Su CY, et al. A broadly neutralizing antibody targets the dynamic hiv envelope trimer apex via a long, rigidified, and anionic β-Hairpin structure. Immunity. 2017;46:690-702.

58. Loos A, Gach JS, Hackl T, et al. Glycan modulation and sulfoengineering of anti-HIV-1 monoclonal antibody PG9 in plants. Proc Natl Acad Sci U S A. 2015;112:12675-80.

59. Hildebrandt H, Dityatev A. Polysialic acid in brain development and synaptic plasticity. Top Curr Chem. 2015;366:55-96.

60. Mercx S, Smargiasso N, Chaumont F, De Pauw E, et al. Inactivation of the beta(1,2)-xylosyltransferase and the alpha(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.