Bioprocess Biosyst Eng
DOI 10.1007/s00449-007-0151-y

MINI REVIEW

Current achievements in the production of complex biopharmaceuticals with moss bioreactors

Eva L. Decker · Ralf Reski

Received: 8 May 2007 / Accepted: 26 July 2007

Abstract Transgenic plants are promising alternatives for the low-cost and safe pathogen-free production of complex recombinant pharmaceutical proteins (molecular farming). Plants as higher eukaryotes perform posttranslational modifications similar to those of mammalian cells. However, plant-specific protein *N*-glycosylation was shown to be immunogenic, a fact that represents a drawback for many plant systems in biopharmaceutical production. The moss *Physcomitrella patens* offers unique properties as a contained system for protein production. It is grown in the predominant haploid gametophytic stage as tissue suspension cultures in photobioreactors. Efficient secretory signals and a transient transfection system allow the secretion of freshly synthesized proteins to the surrounding medium. The key advantage of *Physcomitrella* compared to other plant systems is the feasibility of targeted gene replacements. By this means, moss strains with non-immunogenic humanized glycan patterns were created. Here we present an overview of the relevant aspects for establishing moss as a production system for recombinant biopharmaceuticals.

Keywords Protein glycosylation · Molecular farming · Physcomitrella patens · Biopharmaceuticals · ADCC

Abbreviations

GMP	Good manufacturing practice
ADCC	Antibody-dependent cellular cytotoxicity
HAS	Human serum albumin
VEGF	Vascular endothelial growth factor
CHO	Chinese Hamster Ovary

E. L. Decker (✉) · R. Reski
Plant Biotechnology, Faculty of Biology,
Freiburg University, Schaenzlestr. 1,
79104 Freiburg, Germany
e-mail: eva.decker@biologie.uni-freiburg.de

Introduction

The production of recombinant pharmaceutical proteins began to be developed more than 25 years ago [1, 2] with nowadays more than 300 protein products on the market or in late clinical stages [3]. The vast majority of these proteins have been produced in microbial (e.g., *Escherichia coli*, *Saccharomyces cerevisiae*) or mammalian systems (mainly Chinese Hamster Ovary cells). While microbial systems are clearly superior in terms of ease of handling and high product yields, mammalian cell lines are favored when correct posttranslational modification of the protein target is necessary [3–6]. Compared to these production hosts, plant-based systems combine advantages of the currently used systems making them superior production hosts. As higher eukaryotes plants synthesize complex multimeric proteins with posttranslational modifications closely resembling mammalian modifications. The use of plants eliminates the risk of product contamination by infectious agents deriving from the used cell line or culture media [7, 8]. Agricultural growth offers opportunities for easy and nearly unlimited adaption of production scale and low-cost production. However, natural variations and inconsistencies in growth, soil and weather conditions limit the possibilities to establish current Good Manufacturing Practice (GMP) conditions, indispensable for pharmaceutical production, for field-grown plants [9]. Some of these limitations could be addressed by contained growth in greenhouses alternatively in vitro plant cell or tissue culture offers a precisely controlled environment that allows producing under GMP

conditions [10]. Transformation of many plant species is established and easy to achieve [11]. Evidence for the feasibility of plants as expression hosts for pharmaceutical proteins was produced more than 15 years ago [12, 13]. Meanwhile several plant-derived pharmaceuticals have been submitted for clinical trials in humans and last year the first plant-derived pharmaceutical, a veterinary vaccine produced in plant cell culture, got approval to be released to the market [14]. However, most plant systems are limited in their product spectrum concerning glycosylated proteins. Protein *N*-glycosylation is a common modification found on the majority of proteins from human blood, which are main candidates as biopharmaceuticals (e.g., antibodies, growth factors, cytokines, hormones). Appropriate glycosylation may be important for activity, stability or immunogenicity of a protein [6, 15] or may influence the affinity and effectiveness of certain antibodies (antibody-dependent cellular cytotoxicity—ADCC) [16, 17]. While plants are able to attach glycan structures to proteins which are resembling mammalian glycosylation most of all alternative production systems, plant glycans contain sugar moieties which are immunogenic or were shown to cause allergic reactions in patients [18–22]. A few years ago, the moss *Physcomitrella patens* was suggested and commercialized as an alternative production host that meets this concern by providing an exceptional amenability for precise genetic modifications together with low-cost contained cultivation [23] (http://www.greenovation.com).

Here we summarise basic principles and recent progress in establishing moss as a safe and efficient production system for recombinant biopharmaceuticals.

Moss cultivation

In vitro cultivation of *P. patens* is established for all stages of the mosses life cycle (Fig. 1). It starts with the germination of a haploid spore under sufficient light and water supply resulting in the outgrowth of a cell filament, the so-called protonema, proliferating via apical cell divisions (Fig. 1b). Division of sub-apical protonema cells will provoke the development of branches or buds which subsequently give rise to the adult plant, i.e., the gametophore, with stems, leaflets and rhizoids (Fig. 1d). Under appropriate conditions, the adult moss plant develops sex organs. After self-fertilisation a spore capsule grows on top of the gametophore and with the subsequent release of the spores the life cycle is completed (Fig. 1a). The whole life cycle could be followed by cultivation on agar plates however, most convenient for mass production of plant material for diverse biotechnological approaches is the vegetative propagation of protonema tissue in suspension cultures. Plate cultivation is mainly performed for space-saving storage of different transgenic strains, which should be sub-cultivated once or twice a year (Fig. 1e) [24]. For long-term storage, cryo-conservation protocols were established [25] and realized in the International Moss Stock Centre (http://www.moss-stock-center.org/).

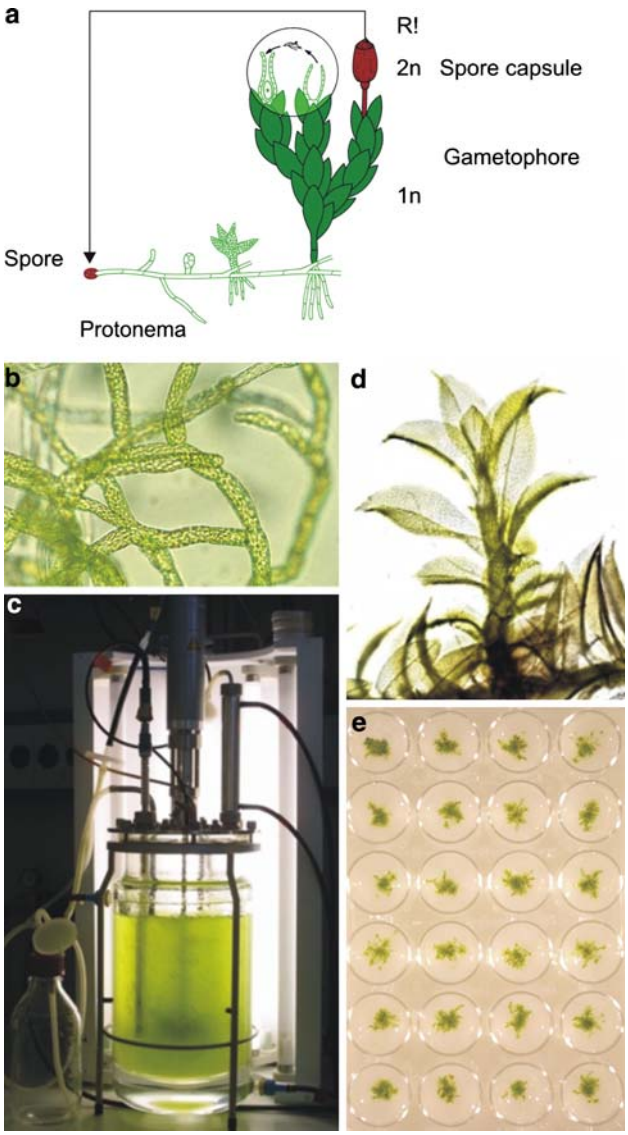

Fig. 1 Moss life cycle and in-vitro cultivation of *P. patens*. **a** Germination of spores results in the outgrowth of filamentous protonema tissue and the subsequent development of the adult gametophore which carries the sex organs and after fertilization the diploid spore capsule, resp. In-vitro propagation of protonema (**b, c**) in photobioreactors and of adult moss plants (**d, e**) on multi-well agar plates. Photographs courtesy of Annette Büttner (**d**) and Andreas Schaaf (**e**), respectively

Cultivation always is performed with the normally growing tissues instead of cell lines derived from callus material or single cells. Therefore, genetic instability, which might occur in de-differentiated cell lines never was observed with moss tissue cultures. The moss grows in a

simple medium of inorganic salts in constant low light or day–night light cycles. As the only necessary carbon source airborne carbon dioxide is provided. For special applications or growth of auxotrophic strains, various additives were tested on their effects of moss growth [26, 27]. The requirement of very weak light and the simplicity of the growth medium make moss cultivation very cost-effective compared to other plant cell cultures or animal and plant cultivation, respectively. Small-scale cultivation is performed in glass flasks agitated on simple shakers. Highly controllable and standardised cultivation conditions can be reached in different photo-bioreactors developed as stirred glass tanks for a volume up to 15l (Fig. 1c) [28, 29]. Parameters for high-density and uniform moss cultures were determined for semi-continuous cultivation as well as for continuous cultivation in a modular, fully scalable glass tubular bioreactor [30]. Future developments in moss bioreactor technology will focus on the improvement of this tubular system by establishing a 100-l photo-bioreactor for the manufacture of recombinant proteins to be used in clinical studies (http://www.greenovation.com).

Cultivation as tissue cultures is not only advantageous in terms of safety of production [10] but also enables simpler and cheaper downstream processing and purification when the pharmaceutical product is secreted from the cells. By adding different stabilizing additives (PVP, HSA) to the moss medium, product amounts could be enhanced [31]. However, enhancing concentrations of these additives interfered with the cultivation process because of foam formation or the purification process by reduced binding of the pharmaceutical product to chromatography columns. Co-expression of recombinant HSA, which is widely used as a protection agent further enhanced recovery of the target protein but avoided the negative effects of the media additives [31]. The secretory moss expression system was also used for transient protein production allowing quick feasibility studies of expression cassettes, transgenic moss strains or expression and purification purposes of a new product of choice [32–37]. The transient system was optimized for high-level expression of recombinant human VEGF (vascular endothelial growth factor) up to 10 µg/ml [38].

Genetic engineering of moss strains for enhanced product yields

The overall used method of choice for transformation of *Physcomitrella patens* is polyethylenglycol-mediated transfection of moss protoplasts. Established protocols for the generation of transgenic moss strains, e.g., [24, 39] were modified in order to build up a transient expression system [38]. After a transfection procedure all in all taking 3 h, harvest and analysis of the recombinant protein product may start only a few hours later and last up to several weeks.

Genetic tools for improving foreign protein production focused on the establishment of promoter sets for flexible expression and the validation of signal sequences for intracellular sorting and secretion (Fig. 2). Various heterologous promoters from plant and mammalian expression systems were quantified for their activity in moss and compared with endogenous promoters [40] among the latter *Physcomitrella* tubulin and actin promoters [36, 37]. From the endogenous promoters, upstream sequences of the moss actin homologue PpAct5 were shown to be strongest in transcriptional activation. In addition, inducible promoter systems were shown to be functional in moss, e.g., the glucocorticoid-inducible gene expression system and tetracycline-repressor system [41, 42] as well as the abscisic acid and osmotic stress-induced wheat Em promoter [43] and the auxin-inducible GH3 and DR5 promoter systems [44]. Recently, a soybean heat-shock promoter was identified as strongly inducible in *Physcomitrella* after heat-shock as well as treatment with acetyl salicylic acid and was suggested for controlled recombinant protein synthesis in moss [45].

Efficient secretion signals are needed when the protein production process relies on a secretory system. Producing complex modified proteins, a secretory system is desirable not only because of ease of downstream processing and purification but also to navigate the emerging proteins through those compartments in eukaryotic cells which are responsible for many posttranslational modifications (e.g., *N*-glycosylation). These are the endoplasmic reticulum and the Golgi apparatus, which are passed by the vast majority of extracellular proteins. In different studies, secretion signals were evaluated for their efficiency in directing target proteins to the extracellular space. While human

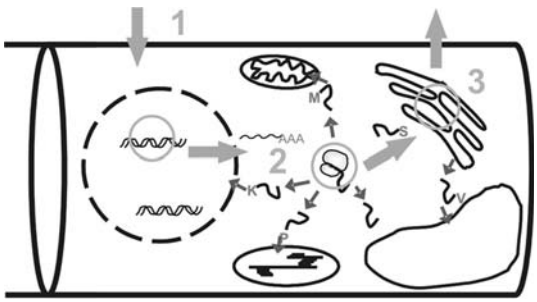

Fig. 2 Optimization of moss strains for recombinant protein production. Schematic drawing of a single moss protonema cell. *1* Cultivation scale and conditions have to be adapted to satisfy the specific requirements of protein production. *2* Genetic engineering of expression constructs provides high-level gene expression and proper intracellular protein sorting. *3* Engineering of posttranslational modifications enhances safety, functionality and efficacy of products

signals were shown to be functional in moss, most plant signals, from heterologous or moss source, turned out to be superior to the native human sequences in secretion of recombinant proteins [35, 37]. In addition to various moss secretion signals, signal sequences for intracellular storage of proteins in chloroplasts, mitochondria or the central vacuole were identified and characterized via GFP reporter fusions [34, 46–48].

Genetic engineering of moss strains for enhanced product quality

All non-human expression hosts for biopharmaceutical proteins synthesize products with slight differences to the native human counterpart. While folding and assembly of multimeric proteins generally is performed in all higher eukaryotes (e.g., plants and mammals) in the same manner, posttranslational modifications differ between species. For this reason, mammalian cell lines are the currently preferred system for the production of glycosylated proteins as their native glycosylation patterns resemble human sugar structures most. Non-mammalian systems should be adaptable to human glycosylation to avoid functional loss or immunogenicity of the produced therapeutics. An appropriate system needs to be genetically well known and amenable for genetic modifications. The moss genome has been sequenced recently (http://www.cosmoss.org) and large amounts of EST data comprise more than 95% of the transcribed moss genes [49–52]. Analysis of these data revealed that codon usage in moss allows expression of human genes without previous codon adaptation, which is necessary in some other hosts, e.g., algae [53]. The genetically most interesting feature of the moss is the high degree of homologous recombination in its nuclear DNA offering a convenient possibility of targeted gene knockouts. As this possibility is still not given for any other plant, the moss was termed "green yeast" [54].

In parallel with the exploitation of the moss transcriptome protein N-glycosylation was characterized and shown to exhibit the same structures as in higher plants [55, 56]. In addition, the glycosyltransferase genes responsible for key steps of complex-type glycosylation were identified and isolated from the moss genome [55].

The most critical differences between plant and human glycosylation were shown to be two plant-specific sugar moieties, a xylose connected to the core mannose residue by beta 1,2-linkage and a fucose, alpha 1,3-linked to the proximal GlcNAc (*N*-acetyl glucosamine) residue of the core glycan. While xylose is a sugar completely unknown from human glycans, the proximal fucose residue in human glycans is linked in a different fashion (alpha 1,6). Both of the plant-specific sugars showed to confer immunogenicity to the glycosylated protein. About one-quarter of individuals with allergies developed antibodies of the allergy-relevant IgE class, which specifically recognize xylose or fucose-containing complex-type glycans [20, 57]. Moss knockout strains were created which lacked the enzymes responsible for xylosylation and fucosylation, beta 1,2-xylosyltransferase and alpha1,3-fucosyltransferase, respectively [58]. The resulting double knockout strains were shown to be completely devoid of the allergenic sugar residues and were employed to synthesize several products of pharmaceutical value, including human VEGF [58], antibodies IgG1 [59] and IgG4 [60], and erythropoietin [61]. For all products analysed so far, over-glycosylation never was detected (G. Gorr, personal communication).

Further "humanization" of plant *N*-glycans comprises expression of a beta 1,4-galactosyltransferase, responsible for linking terminal galactose to mammalian *N*-glycans and lacking in plant genomes [62], [63]. This human-like galactosylation was also realised by "knockin" of the human galactosyltransferase gene in the xylosyltransferase and fucosyltransferase locus, respectively, from double knockout moss strains [32, 60].

The above mentioned glycan modifications already shifted the plant complex-type glycosylation very close to the human type. Still missing is the engineering of plant genomes in order to attach sialyl residues to the very ends of sugar chains as plants donot synthesize sialic acids [64]. While there was a report about detection of sialic acids in the model plant *Arabidopsis thaliana* [65] follow-up study proved that these were likely to be contaminations [66]. Sialic acid residues are linked to the very end of some human blood proteins, e.g., erythropoietin, and enhance their half-life in the blood circulation [67, 68]. However, antibodies, comprising the probably most interesting and largest group of biotech drugs in use and clinical development [69] have one conserved glycosylation site and show sialylation of only a small fraction of the attached *N*-glycan. Moreover, CHO cells, standard system for production of therapeutical antibodies, tend to undersialylate the recombinant protein [61]. Therefore, plant-based antibody production with appropriate glycosylation is a realizable task, which was demonstrated for moss recently [60].

Moreover, by glyco-engineering via targeted gene knockouts the quality of plant-produced antibodies can even be better than with traditional production hosts. The pharmacological efficiency of some antibodies was shown to be rather disappointing due to weak antibody-dependent cellular cytotoxicity (ADCC), an important effector function of antibodies. ADCC is mediated by receptor (FcgammaRIII) binding of IgG antibodies. The IgG–receptor-binding

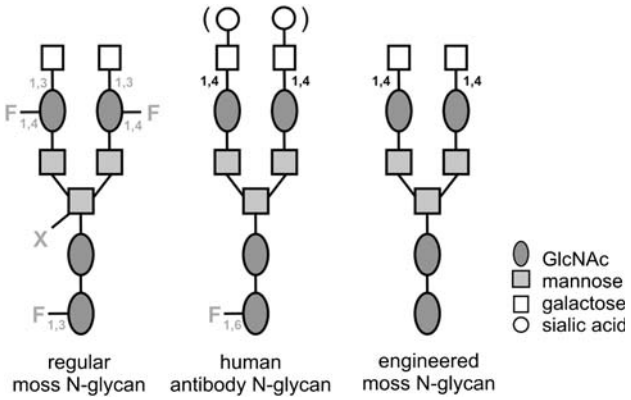

Fig. 3 Typical structures of antibody N-glycosylation. The sugar chains are linked to the amino acid sequence via the proximal GlcNAc residue. Unmodified moss glycan containing terminal galactose and fucose residues (*left*). Typical bisecting human antibody glycan (*middle*). Glyco-optimized moss *N*-glycan providing antibodies with improved ADCC (*right*). *F* fucose; *X* xylose. Sugars or linkages that may result in immunogenicity or low effector function are marked in *grey*

affinity was found to be increased in the absence of the human core fucose residue linked to the IgG *N*-glycan. Increased ADCC of recombinant antibodies produced in glyco-engineered plants was demonstrated for the aquatic plant *Lemna minor* as well as for *Physcomitrella* [59, 70]. A recombinant IgG1 antibody, produced in glyco-optimized moss strains (with missing core fucose) resulted in 40-fold enhanced ADCC activity compared to the parental antibody produced in mammalian cells [59], impressively demonstrating the suitability of moss to produce biopharmaceuticals with enhanced product quality (Fig. 3).

Conclusions

Moss bioreactors offer a safe and efficient scalable system to produce complex modified recombinant pharmaceuticals. Genome engineering and characterization of transgenic strains is eased by the recently finished moss genome sequencing. Optimization of culture conditions and genetic engineering of production lines via targeted gene replacement helped to enhance product yields and safety. Improved antibody function via glycan optimization makes the system even advantageous over currently used mammalian-based production systems disclosing the possibility to establish moss as an alternative system to currently used mammalian-based production.

Acknowledgments This work was supported by the German Federal Ministry of Education and Research (BMBF grants 0312624 and 0313852), the German Academic Exchange Service (DAAD), and the Wissenschaftliche Gesellschaft of the University of Freiburg.

References

1. Goeddel DV, Kleid DG, Bolivar F, Heyneker HL, Yansura DG, Crea R, Hirose T, Kraszewski A, Itakura K, Riggs AD (1979) Expression in *Escherichia coli* of chemically synthesized genes for human insulin. Proc Natl Acad Sci USA 76:106–110
2. Martial JA, Hallewell RA, Baxter JD, Goodman HM (1979) Human growth hormone: complementary DNA cloning and expression in bacteria. Science 205:602–607
3. Schmidt FR (2004) Recombinant expression systems in the pharmaceutical industry. Appl Microbiol Biotechnol 65:363–372
4. Andersen DC, Krummen L (2002) Recombinant protein expression for therapeutic applications. Curr Opin Biotechnol 13: 117–123
5. Butler M (2005) Animal cell cultures: recent achievements and perspectives in the production of biopharmaceuticals. Appl Microbiol Biotechnol 68:283–291
6. Walsh G, Jefferis R (2006) Post-translational modifications in the context of therapeutic proteins. Nat Biotechnol 24:1241–1252
7. Fischer R, Stoger E, Schillberg S, Christou P, Twyman RM (2004) Plant-based production of biopharmaceuticals. Curr Opin Plant Biol 7:152–158
8. Ma JK, Drake PM, Christou P (2003) The production of recombinant pharmaceutical proteins in plants. Nat Rev Genet 4:794–805
9. Ma JK, Chikwamba R, Sparrow P, Fischer R, Mahoney R, Twyman RM (2005) Plant-derived pharmaceuticals—the road forward. Trends Plant Sci 10:580–585
10. Hellwig S, Drossard J, Twyman RM, Fischer R (2004) Plant cell cultures for the production of recombinant proteins. Nat Biotechnol 22:1415–1422
11. Schillberg S, Twyman RM, Fischer R (2005) Opportunities for recombinant antigen and antibody expression in transgenic plants-technology assessment. Vaccine 23:1764–1769
12. Sijmons PC, Dekker BM, Schrammeijer B, Verwoerd TC, van den Elzen PJ, Hoekema A (1990) Production of correctly processed human serum albumin in transgenic plants. Biotechnology (NY) 8:217–221
13. During K, Hippe S, Kreuzaler F, Schell J (1990) Synthesis and self-assembly of a functional monoclonal antibody in transgenic *Nicotiana tabacum*. Plant Mol Biol 15:281–293
14. Walsh G (2006) Biopharmaceutical benchmarks 2006. Nat Biotechnol 24:769–776
15. Schellekens H (2002) Bioequivalence and the immunogenicity of biopharmaceuticals. Nat Rev Drug Discov 1:457–462
16. Shields RL, Lai J, Keck R, O'Connell LY, Hong K, Meng YG, Weikert SH, Presta LG (2002) Lack of fucose on human IgG1 N-linked oligosaccharide improves binding to human Fcgamma RIII and antibody-dependent cellular toxicity. J Biol Chem 277:26733–26740
17. Shinkawa T, Nakamura K, Yamane N, Shoji-Hosaka E, Kanda Y, Sakurada M, Uchida K, Anazawa H, Satoh M, Yamasaki M, Hanai N, Shitara K (2003) The absence of fucose but not the presence of galactose or bisecting *N*-acetylglucosamine of human IgG1 complex-type oligosaccharides shows the critical role of enhancing antibody-dependent cellular cytotoxicity. J Biol Chem 278:3466–3473
18. Bardor M, Faveeuw C, Fitchette AC, Gilbert D, Galas L, Trottein F, Faye L, Lerouge P (2003) Immunoreactivity in mammals of two typical plant glyco-epitopes, core alpha(1,3)-fucose and core xylose. Glycobiology 13:427–434
19. Garcia-Casado G, Sanchez-Monge R, Chrispeels MJ, Armentia A, Salcedo G, Gomez L (1996) Role of complex asparagine-linked glycans in the allergenicity of plant glycoproteins. Glycobiology 6:471–477

20. van Ree R, Cabanes-Macheteau M, Akkerdaas J, Milazzo JP, Loutelier-Bourhis C, Rayon C, Villalba M, Koppelman S, Aalberse R, Rodriguez R, Faye L, Lerouge P (2000) Beta(1,2)-xylose and alpha(1,3)-fucose residues have a strong contribution in IgE binding to plant glycoallergens. J Biol Chem 275:11451–11458
21. Westphal S, Kolarich D, Foetisch K, Lauer I, Altmann F, Conti A, Crespo JF, Rodriguez J, Enrique E, Vieths S, Scheurer S (2003) Molecular characterization and allergenic activity of Lyc e 2 (beta-fructofuranosidase), a glycosylated allergen of tomato. Eur J Biochem 270:1327–1337
22. Faye L, Boulaflous A, Benchabane M, Gomord V, Michaud D (2005) Protein modifications in the plant secretory pathway: current status and practical implications in molecular pharming. Vaccine 23:1770–1778
23. Decker EL, Reski R (2004) The moss bioreactor. Curr Opin Plant Biol 7:166–170
24. Frank W, Decker EL, Reski R (2005) Molecular tools to study *Physcomitrella patens*. Plant Biol 7:220–227
25. Schulte J, Reski R (2004) High throughput cryopreservation of 140,000 *Physcomitrella patens* mutants. Plant Biol 6:119–127
26. Egener T, Granado J, Guitton MC, Hohe A, Holtorf H, Lucht JM, Rensing SA, Schlink K, Schulte J, Schween G, Zimmermann S, Duwenig E, Rak B, Reski R (2002) High frequency of phenotypic deviations in *Physcomitrella patens* plants transformed with a gene-disruption library. BMC Plant Biol 2:6
27. Schween G, Hohe A, Koprivova A, Reski R (2003) Effects of nutrients, cell density and culture techniques on protoplast regeneration and early protonema development in a moss, *Physcomitrella patens*. J Plant Physiol 160:209–212
28. Hohe A, Decker EL, Gorr G, Schween G, Reski R (2002) Tight control of growth and cell differentiation in photoautotrophically growing moss (*Physcomitrella patens*) bioreactor cultures. Plant Cell Rep 20:1135–1140
29. Hohe A, Reski R (2002) Optimisation of a bioreactor culture of the moss *Physcomitrella patens* for mass production of protoplasts. Plant Sci 163:69–74
30. Lucumi A, Posten C (2006) Establishment of long-term perfusion cultures of recombinant moss in a pilot tubular photobioreactor. Proc Biochem 41:2180–2187
31. Baur A, Reski R, Gorr G (2005) Enhanced recovery of a secreted recombinant human growth factor using stabilizing additives and by co-expression of human serum albumin in the moss *Physcomitrella patens*. Plant Biotechnol J 3:331–340
32. Huether CM, Lienhart O, Baur A, Stemmer C, Gorr G, Reski R, Decker EL (2005) Glyco-engineering of moss lacking plant-specific sugar residues. Plant Biol 7:292–299
33. Koprivova A, Stemmer C, Altmann F, Hoffmann A, Kopriva S, Gorr G, Reski R, Decker EL (2004) Targeted knockouts of *Physcomitrella* lacking plant-specific immunogenic *N*-glycans. Plant Biotech J 2:517–523
34. Schaaf A, Reski R, Decker EL (2004) A novel aspartic proteinase is targeted to the secretory pathway and to the vacuole in the moss Physcomitrella patens. Eur J Cell Biol 83:145–152
35. Schaaf A, Tintelnot S, Baur A, Reski R, Gorr G, Decker EL (2005) Use of endogenous signal sequences for transient production and efficient secretion by moss (*Physcomitrella patens*) cells. BMC Biotechnol 5:30
36. Jost W, Link S, Horstmann V, Decker EL, Reski R, Gorr G (2005) Isolation and characterisation of three moss-derived beta-tubulin promoters suitable for recombinant expression. Curr Genet 47:111–120
37. Weise A, Rodriguez-Franco M, Timm B, Hermann M, Link S, Jost W, Gorr G (2006) Use of *Physcomitrella patens* actin 5′ regions for high transgene expression: importance of 5′ introns. Appl Microbiol Biotechnol 70:337–345
38. Baur A, Kaufmann F, Rolli H, Weise A, Luethje R, Berg B, Braun M, Baeumer W, Kietzmann M, Reski R, Gorr G (2005) A fast and flexible PEG-mediated transient expression system in plants for high level expression of secreted recombinant proteins. J Biotechnol 119:332–342
39. Strepp R, Scholz S, Kruse S, Speth V, Reski R (1998) Plant nuclear gene knockout reveals a role in plastid division for the homolog of the bacterial cell division protein FtsZ, an ancestral tubulin. Proc Natl Acad Sci USA 95:4368–4373
40. Horstmann V, Huether CM, Jost W, Reski R, Decker EL (2004) Quantitative promoter analysis in Physcomitrella patens: a set of plant vectors activating gene expression within three orders of magnitude. BMC Biotechnol 4:13
41. Schaefer DG (2002) A new moss genetics: targeted mutagenesis in *Physcomitrella patens*. Annu Rev Plant Biol 53:477–501
42. Zeidler M, Gatz C, Hartmann E, Hughes J (1996) Tetracycline-regulated reporter gene expression in the moss *Physcomitrella patens*. Plant Mol Biol 30:199–205
43. Knight CD, Sehgal A, Atwal K, Wallace JC, Cove DJ, Coates D, Quatrano RS, Bahadur S, Stockley PG, Cuming AC (1995) Molecular responses to abscisic acid and stress are conserved between moss and cereals. Plant Cell 7:499–506
44. Bierfreund NM, Reski R, Decker EL (2003) Use of an inducible reporter gene system for the analysis of auxin distribution in the moss *Physcomitrella patens*. Plant Cell Rep 21:1143–1152
45. Saidi Y, Finka A, Chakhporanian M, Zryd JP, Schaefer DG, Goloubinoff P (2005) Controlled expression of recombinant proteins in *Physcomitrella patens* by a conditional heat-shock promoter: a tool for plant research and biotechnology. Plant Mol Biol 59:697–711
46. Kiessling J, Kruse S, Rensing SA, Harter K, Decker EL, Reski R (2000) Visualization of a cytoskeleton-like FtsZ network in chloroplasts. J Cell Biol 151:945–950
47. Kiessling J, Martin A, Gremillon L, Rensing SA, Nick P, Sarnighausen E, Decker EL, Reski R (2004) Dual targeting of plastid division protein FtsZ to chloroplasts and the cytoplasm. EMBO Rep 5:889–894
48. Richter U, Kiessling J, Hedtke B, Decker E, Reski R, Borner T, Weihe A (2002) Two RpoT genes of *Physcomitrella patens* encode phage-type RNA polymerases with dual targeting to mitochondria and plastids. Gene 290:95–105
49. Nishiyama T, Fujita T, Shin IT, Seki M, Nishide H, Uchiyama I, Kamiya A, Carninci P, Hayashizaki Y, Shinozaki K, Kohara Y, Hasebe M (2003) Comparative genomics of *Physcomitrella patens* gametophytic transcriptome and *Arabidopsis thaliana*: implication for land plant evolution. Proc Natl Acad Sci USA 100:8007–8012
50. Rensing SA, Rombauts S, Van de Peer Y, Reski R (2002) Moss transcriptome and beyond. Trends Plant Sci 7:535–538
51. Lang D, Eisinger J, Reski R, Rensing SA (2005) Representation and high-quality annotation of the *Physcomitrella patens* transcriptome demonstrates a high proportion of proteins involved in metabolism in mosses. Plant Biol 7:238–250
52. Rensing SA, Fritzowsky D, Lang D, Reski R (2005) Protein encoding genes in an ancient plant: analysis of codon usage, retained genes and splice sites in a moss, *Physcomitrella patens*. BMC Genomics 6:43
53. Franklin SE, Mayfield SP (2004) Prospects for molecular farming in the green alga *Chlamydomonas*. Curr Opin Plant Biol 7:159–165
54. Schaefer DG (2001) Gene targeting in *Physcomitrella patens*. Curr Opin Plant Biol 4:143–150
55. Koprivova A, Altmann F, Gorr G, Kopriva S, Reski R, Decker EL (2003) N-Glycosylation in the moss *Physcomitrella patens* is organized similarly to higher plants. Plant Biol 5:582–591

56. Vietor R, Loutelier-Bourhis C, Fitchette AC, Margerie P, Gonneau M, Faye L, Lerouge P (2003) Protein N-glycosylation is similar in the moss *Physcomitrella patens* and in higher plants. Planta 218:269–275
57. Mari A (2002) IgE to cross-reactive carbohydrate determinants: analysis of the distribution and appraisal of the in vivo and in vitro reactivity. Int Arch Allergy Immunol 129:286–295
58. Koprivova A, Stemmer C, Altmann F, Hoffmann A, Kopriva S, Gorr G, Reski R, Decker EL (2004) Targeted knockouts of *Physcomitrella* lacking plant-specific immunogenic *N*-glycans. Plant Biotechnol J 2:517–523
59. Nechansky A, Schuster M, Jost W, Siegl P, Wiederkum S, Gorr G, Kircheis R (2007) Compensation of endogenous IgG mediated inhibition of antibody-dependent cellular cytotoxicity by glycoengineering of therapeutic antibodies. Mol Immunol 44:1826–1828
60. Gorr G, Jost W (2005) Glycosylation design in transgenic moss for better product efficacy. Bioprocess J 4:26–30
61. Gorr G, Altmann F (2006) Glycosylation of recombinant proteins in plants. In: Kayser O, Quax W (eds) Medical plant biotechnology. Wiley-VCH, Weinheim, pp 345–374
62. Bakker H, Bardor M, Molthoff JW, Gomord V, Elbers I, Stevens LH, Jordi W, Lommen A, Faye L, Lerouge P, Bosch D (2001) Galactose-extended glycans of antibodies produced by transgenic plants. Proc Natl Acad Sci USA 98:2899–2904
63. Palacpac NQ, Yoshida S, Sakai H, Kimura Y, Fujiyama K, Yoshida T, Seki T (1999) Stable expression of human beta1,4-galactosyltransferase in plant cells modifies N-linked glycosylation patterns. Proc Natl Acad Sci USA 96:4692–4697
64. Lerouge P, Bardor M, Pagny S, Gomord V, Faye L (2000) *N*-glycosylation of recombinant pharmaceutical glycoproteins produced in transgenic plants: towards an humanisation of plant *N*-glycans. Curr Pharm Biotechnol 1:347–354
65. Shah MM, Fujiyama K, Flynn CR, Joshi L (2003) Sialylated endogenous glycoconjugates in plant cells. Nat Biotechnol 21:1470–1471
66. Zeleny R, Kolarich D, Strasser R, Altmann F (2006) Sialic acid concentrations in plants are in the range of inadvertent contamination. Planta 224:222–227
67. Delorme E, Lorenzini T, Giffin J, Martin F, Jacobsen F, Boone T, Elliott S (1992) Role of glycosylation on the secretion and biological activity of erythropoietin. Biochemistry 31:9871–9876
68. Erbayraktar S, Grasso G, Sfacteria A, Xie QW, Coleman T, Kreilgaard M, Torup L, Sager T, Erbayraktar Z, Gokmen N, Yilmaz O, Ghezzi P, Villa P, Fratelli M, Casagrande S, Leist M, Helboe L, Gerwein J, Christensen S, Geist MA, Pedersen LO, Cerami-Hand C, Wuerth JP, Cerami A, Brines M (2003) Asialoerythropoietin is a nonerythropoietic cytokine with broad neuroprotective activity in vivo. Proc Natl Acad Sci USA 100:6741–6746
69. Ma JK, Barros E, Bock R, Christou P, Dale PJ, Dix PJ, Fischer R, Irwin J, Mahoney R, Pezzotti M, Schillberg S, Sparrow P, Stoger E, Twyman RM (2005) Molecular farming for new drugs and vaccines. Current perspectives on the production of pharmaceuticals in transgenic plants. EMBO Rep 6:593–599
70. Cox KM, Sterling JD, Regan JT, Gasdaska JR, Frantz KK, Peele CG, Black A, Passmore D, Moldovan-Loomis C, Srinivasan M, Cuison S, Cardarelli PM, Dickey LF (2006) Glycan optimization of a human monoclonal antibody in the aquatic plant *Lemna minor*. Nat Biotechnol 24:1591–1597