COST Action FA0804

Molecular Farming: Plant as a Production Platform for High Value Proteins

WG2 and WG 3 Meeting

Programme and available abstracts

Hotel Wageningse Berg, Wageningen, Nederland
January 25-26, 2010

Local organizers:
Dirk Bosch, WUR, Netherlands
Arjen Schots, WUR, Netherlands

Tentative Programme WG2 and WG3 COST Action FAO804 Molecular farming
Focus: Downstream processing and Facilities

Monday January 25

08.00-08.30	Registration
08.30-08.45	Introduction Kirsi Marja Oksman
08.45-09.30	Patrick van Berkel (Genmab, Utrecht, NL): Production platform assessment for biopharmaceutical proteins
09.30-10.15	Juergen Drossard (Fraunhofer IME, Aachen, D): Technical and regulatory developments in PMP production – lessons learned from Pharma-Planta
10.15-10.45	Coffee Break
10.45-11.45	Silke Hemming (Wageningen UR, Wageningen, NL): Efficient crop production in controlled greenhouses
11.45-12.30	Erik Pekkeriet (Wageningen UR, Wageningen, NL): Automation in plant handling and monitoring
12.30-13.45	Lunch
13.45-14.15	Ronald Bassuner (Greenovation Biotech GmbH, Freiburg, D) tba
14.15-14.45	Udo Conrad (IPK, Gatersleben, D) Purification of ELPylated proteins from plants by Inverse Transition Cycling: antigens, antibodies and spider silk proteins
15.00-15.30	Bus to Wageningen UR facilities
15.30-17.30	Tour Greenhouse facilities Wageningen UR
18.00-23.00	Dinner (by bus)

Tuesday January 26

PhD session	(20 minutes presentations by PhDs and PostDocs)
08.30-08.50	Luisa Bortesi, Verona: Interleukin-10: targeting\transient\stable
08.50-09.10	Petya Stoykova, Sofia: Expression of human acidic fibroblast growth factor in tomato *Solanum lycopersicum*
09.10-09.30	Stefanie Goedeke, Gatersleben: Production of Recombinant Protein in Transgenic Barley Grains
09.30-09.50	Eva Thuenemann, Norwich: Transient Expression of Complex Heteromeric Bluetongue Virus-like Particles
09.50-10.10	Withdrawn
10.10-10.40	Coffee break
10.40-11.00	Lotte Westerhof, Wageningen: Aggregation as a Bottleneck for IL-10 Production in *Nicotiana benthamiana*
11.00-11.20	Inge Broer, Rostock: Biopolymers in Transgenic Plants: Optimization of Cyanophycin Production in Different Species
11.20-12.00	Dirk Bosch & Arjen Schots,Wageningen – Stefan Schillberg, Aachen Interactive database: presentation and discussion
12.15-13.30	Lunch
13.30-13.50	Kirsten de Wilde, Gent: Inter-transformant transgene expression variability in *Arabidopsis* leaves and seeds.
13.50-14.10	Michele Belluci, Perugia: Enzyme Replacement Therapy: Production of Human α-Mannosidase in Transgenic Tobacco Plants
14.10-15.00	Wrap up and general discussion

15.00 Closure

Enzyme Replacement Therapy: Production of Human α-Mannosidase in Transgenic Tobacco Plants

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Abstract

α-Mannosidosis is a rare lysosomal storage disease with autosomal recessive inheritance that leads to mental and physical deterioration. This pathology is due to progressive accumulation of undegraded oligosaccharides inside lysosomes. The deficiency of the α-mannosidase (LAMAN) enzyme, which normally cleaves α-linked mannose residues from glycoproteins during their ordered degradation, is the cause of the disease. The enzyme contains 1011 amino acids (108 kDa), including the 49 N-terminal residues which constitute the signal peptide. It is synthesised as a single chain precursor and sorted to the lysosomes where is processed into three glycopeptides of 70, 42 and 15 kDa. In humans, the 70 kDa peptide is further partially proteolysed into three more peptides that are joined by disulfide bridges.
The objective of this study is to provide a plant-based method for the production of LAMAN to be used in “enzyme replacement therapy” (ERT). We report the expression of the human α-mannosidase gene in stable transformed tobacco plants. Two different constructs were produced: in the first one, pROK8-LAMAN, the full-length cDNA coding sequence of α-mannosidase was used. The gene was under the control of the rbcS (rubisco small subunit) promoter and NOS (nopaline synthase) terminator. In the second one, pGreen-LAMAN, the α-mannosidase cDNA expression was controlled by the CaMV 35S (cauliflower mosaic virus) promoter and terminator. Moreover, the original 49 N-terminal signal peptide was replaced by a specific plant signal peptide from PR1 protein and the FLAG epitope was added at the C-terminus of the protein.
Even if the α-mannosidase gene harboured by the transgenic pROK8-LAMAN tobacco plants showed a good transcription efficiency, no detectable levels of the corresponding enzyme were obtained both in Western blot and enzymatic assays.
On the contrary, pGreen-LAMAN tobacco transformed plants expressed the α-mannosidase enzyme. The main signal detected in Western blot experiments using the anti-FLAG antibody had a molecular mass of about 110 kDa corresponding to the entire protein precursor, indicating that the protein was correctly synthesised. Western blot experiments using antibodies specific for the α-mannosidase enzyme revealed several signals corresponding to the single chain precursor and glycopeptides derived from precursor proteolysis. Transformed plants expressing the protein exhibited an enzymatic activity significantly higher then the untransformed tobacco plants. The recombinant enzyme showed biochemical features comparable to those of the human enzyme. Our long-term goal is to offer a new therapeutic approach for α-mannosidosis.

PRODUCTION OF INTERLEUKIN-10 IN TOBACCO

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ABSTRACT

Interleukin-10 (IL-10) is a potent anti-inflammatory cytokine, with therapeutic applications in several autoimmune and inflammatory diseases. Oral administration of this cytokine, alone or in combination with disease-associated autoantigens, could confer protection form the onset of a specific autoimmune disease through the induction of oral tolerance.

Transgenic plants are attractive systems for production of therapeutic proteins because of the ability to do large scale-up at low cost, and the low maintenance requirements. They are highly amenable to oral administration and could become effective delivery systems without extensive protein purification.

The ability of tobacco plants to produce high levels of biologically-active viral and murine IL-10 was investigated. To reach high accumulation levels of the transgenes, plastid transformation of the IL-10 genes as well as different targeting strategies of the nuclear encoded recombinant proteins were investigated. Chloroplast transformation turned out not to be a feasible approach for the recombinant production of IL-10, as unsatisfactory accumulation levels were obtained upon expression of both transgenes.

For tobacco nuclear transformation, three different subcellular targeting strategies, directing the recombinant protein into the endoplasmic reticulum (ER), cytosol and apoplast, were first assessed in transient expression experiments, and stable transgenic plants were then generated with the constructs that yielded the highest accumulation levels by targeting the recombinant proteins to the ER. The recombinant proteins were purified from transgenic leaf material and characterized in terms of their *N*-glycan composition, dimerization, stability and biological activity in *in vitro* assays. Both molecules formed stable dimers, were able to activate the IL-10 signaling pathway and to induce specific anti-inflammatory responses in mouse J774 macrophage cells.

It was therefore demonstrated that tobacco plants are able to correctly process viral and murine IL-10 into biologically active dimers, representing a suitable platform for the production for these cytokines. The accumulation levels obtained are high enough to allow delivery of an immunologically relevant dose of IL-10 in a reasonable amount of leaf material, without extensive purification. This study paves the way to performing feeding studies in mouse models of autoimmune diseases, that will allow evaluation of the immunomodulatory properties and effectiveness of the viral and murine IL-10 in inducing oral tolerance.

Biopolymers in transgenic plants: Optimization of Cyanophycin production in different species

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ABSTRACT

The production of biodegradable polymers, that substitute petrochemical compounds in commercial products, in transgenic plants is an important challenge for plant biotechnology. The polymer Polyaspartate is used to substitute polycarboxylates. It can be isolated from the bacterial storage protein cyanophycin, composed of L-Aspartat and L-Arginin, hence besides polyaspartate, Arginin is an even greater additional added value. Cyanophycin is produced via non-ribosomal protein biosynthesis by a cyanophycin synthetase. The production of Cyanophycin in plants can be favourable, if the plants allow a cost effective manufacture as a by product to primary plant products like starch, sugar or even nicotine Therefore the cyanophycin synthetase was primarily expressed in tobacco and potato.. While the production of cyanophycin in the cytoplasm of tobacco and potato plants only led to an accumulation up to 0.1 % polymer in dry weight (dw) accompanied by slight stress symtoms, the transfer of the synthetase to the chloroplasts led to a cyanophycin content that increased up to 3 % in dw. Additionally, the plastidic expression did not lead to any phenotypic damage except a slightly thicker cell wall. The reduction of polymer synthesis to potato tuber cytoplasm results in very small tubers with a polymer content up to 2 % in dw, whereas the production of cyanophycin exclusively in the amyloplast of potato tubers led to an increase in cyanophycin content up to 7.5 % dw without a significant reduction in starch contend and yield. Although some of the tubers had brown spots after harvest, the cyanophycin contend was stable over an eight month storage period and the subsequent sprouting was undisturbed. Hence a commercial use of these potatoes is not influenced by the stress symptoms. Nevertheless it remains to be ascertained whether other species are even more suitable for cyanophycin production.

Purification of ELPylated proteins from plants by Inverse Transition Cycling: Antigens, Antibodies and Spider Silk Proteins

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ABSTRACT

Elastin-like polypeptides (ELPs) derived from mammalian elastin are highly biocompatible. They exhibit a thermally responsive reversible phase transition. These properties make ELPs attractive to improve the efficiency with which recombinant proteins can be purified. Fusion of proteins with ELPs ("ELPylation") causes fusion protein inheriting the reversible phase transition property, but still keep their original functionality. ELPylation technology has recently been extended to plant cells. A number of plant-based expression systems have been evaluated for the production of ELPylated proteins.[1].

The ELPylation strategy has been applied to enhance the expression of several antigens and to optimize their enrichement and purification. Examples as a tuberculosis antigen [2] and several bird flu antigens are mentioned as examples.

Two ELPylated ((Val-Pro-Gly-Xaa-Gly)$_{100}$) antibodies expressing a neutralizing capacity against human immunodeficiency virus 1 (HIV-1) were successfully expressed in tobacco leaves and seeds [3,4]. ELPylation resulted in a more efficient recombinant protein accumulation of the heavy and the light chain of both antibodies. ELPylation did not affect the binding affinity, but a slightly negative effect on HIV-neutralization capacity in dependence on fusion to the light chain was detetected. ÉLPylation had no effect on folding and assembly.

Spider silk protein-ELP fusions have also been used to enhance expression and purification and to allow the increase of the moleculary weight by post-translational modifications.

References

1. Floss et al., *Elastic-like polypeptides revolutionize recombinant protein expression and their application in medicine and nanobiotechnology.* Trends in Biotechnology, 2009, oi:10.1016/j.tibtech.2009.10.004.
2. Frutos et al., *Pharmaceutical proteins in plants: a strategic genetic engineering approach for the production of tuberculosis antigens.* Ann. N. Y. Acad. Sci. 1149 (2008) 275-280.
3. Floss, D.M. et al., *Biochemical and functional characterization of anti-HIV antibody-ELP fusion proteins from transgenic plants.* Plant Biotechnol. J. 6, (2008) 379-391.
4. Floss, D.M. *et al.*, *Influence of ELP fusions on the quantity and quality of a tobacco-derived HIV-neutralizing antibody.* Plant Biotechnol. J. (2009) doi:10.1111/**j**.1467-7652.2009.00452.x.

Inter-transformant transgene expression variability in *Arabidopsis* leaves and seeds

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ABSTRACT

Among the many plant-based production systems that are being tested for molecular farming, seeds are very attractive as they provide a stable environment in which the accumulating recombinant proteins can be stored. Using the regulatory sequences of the seed storage protein genes *arcelin 5-I* and *β-phaseolin* of *Phaseolus spp*., the seed-specific accumulation of a model single chain Fv (scFv) recombinant antibody fragment in transgenic homozygous *Arabidopsis thaliana* seeds boosted to exceptionally high levels, reaching 7% of the total seed weight (De Jaeger *et al*., 2002). Also high accumulation levels of more complex scFv-Fc antibodies, corresponding to 2% of seed weight, were achieved using the same expression cassette in *Arabidopsis* seeds (Van Droogenbroeck *et al*., 2007). Strikingly, very low plant-to-plant variation in transgene expression levels were observed in the seeds. This is in contrast to the high inter-transformant expression variability of P35S driven genes in *Arabidopsis* leaves. As the variability of these genes was significantly lower in RNA-dependent-RNA polymerase 6 mutants (*rdr6*) it could be concluded that posttranscriptional gene silencing was the major cause for the high transgene expression variability in leaves (Butaye *et al*., 2004). Better knowledge on construct organization can be the key to a more robust plant-based protein production system. Therefore the aim of our work is to investigate 1) whether plant-to-plant expression variation in 35S driven transgenes is tissue-dependent, 2) whether the variability in seeds for two expression cassettes, driven by the 35S and Phas promoter on the same T-DNA, is correlated or not, 3) whether the *rdr6*-mediated silencing pathway is involved in the variability in seeds and 4) whether the position of the transgene on the T-DNA affects the expression variability in leaves and seeds. To answer these questions we compared GUS activity levels in leaves and seeds of Col-0 and *sgs*2 *Arabidopsis* plants transformed with T-DNA's harbouring a pPhas driven scFv-Fc antibody gene and a P35S driven *gus* gene located internally or near the right border. The results and progress of the project will be presented.

References

1. Butaye, K. *et al*. (2004) *Stable high-level transgene expression in Arabidopsis thaliana using gene silencing mutants and matrix attachment regions*. Plant Journal 39: 440-449
2. De Jaeger, G. *et al.* (2002) *Boosting heterologous protein production in transgenic dicotyledonous seeds using Phaseolus vulgaris regulatory sequences*. Nature biotechnology, 20, 1265-1268.
3. Van Droogenbroeck, B. *et al.* (2007) *Aberrant localization and underglycosylation of highly accumulating single-chain Fv-Fc antibodies in transgenic Arabidopsis seeds*. Proceedings of the National Academy of Sciences of the United States of America, 104, 1430-1435.

Transient Expression of Complex Heteromeric Bluetongue Virus-like Particles

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ABSTRACT

A vector system based on a deleted version of Cowpea Mosaic Virus (CPMV) RNA-2 (delRNA-2) has allowed easy and rapid transient production of heterologous proteins in plants [1-2]. Recently, the original system has been modified, resulting in the production of "hypertranslatable" delRNA-2 molecules which can direct the synthesis of very high levels (up to 30% TSP) of heterologous proteins in agroinoculated leaves. This system, termed CPMV-*HT* has proved very useful for the production of a number of proteins, including virus-like particles (VLPs) and full-size IgG molecules within a few days [3]. In order to achieve efficient simultaneous expression of the structural proteins of Bluetongue Virus (Reoviridae family), however, the existing vector system had to be streamlined.

To increase versatility and user-friendliness of the CPMV-*HT* system, we have developed the pEAQ series of vectors [4]. These have a vector backbone of reduced size and allow one-step cloning of a gene of interest either with or without an N- or C-terminal His-tag. Furthermore the sequence of a suppressor of gene silencing, P19, is also incorporated. The pEAQ vectors are tailor-made for transient expression and can accommodate several genes of interest on the same plasmid. This ensures that T-DNA transfer from a single *Agrobacterium tumefaciens* inoculum will give rise to efficient co-expression of all the genes within a given plant cell.

The *pEAQ* vectors have been used to transiently express and assemble Bluetongue Virus (BTV) core-like particles (two proteins) and virus-like particles (four proteins) in *N.benthamiana*. We have found that the complex stoichiometry of the BTV particles requires modulation of the relative amounts of the proteins produced, in order to achieve efficient assembly of full particles. The speed, simplicity and versatility of the *pEAQ* vectors and the CPMV-*HT* system make them a very useful platform for the development and production of biopharmaceuticals in plants in the future.

References

1. Canizares, M.C., et al., *A bipartite system for the constitutive and inducible expression of high levels of foreign proteins in plants.* Plant Biotechnol J, 2006. **4**(2): p. 183-93.
2. Sainsbury, F., et al., *Expression of multiple proteins using full-length and deleted versions of cowpea mosaic virus RNA-2.* Plant Biotechnol J, 2008. **6**(1): p. 82-92.
3. Sainsbury, F. and G.P. Lomonossoff, *Extremely High-Level and Rapid Transient Protein Production in Plants without the Use of Viral Replication.* Plant Physiol, 2008.
4. Sainsbury, F., E.C. Thuenemann, and G.P. Lomonossoff, *pEAQ: versatile expression vectors for easy and quick transient expression of heterologous proteins in plants.* Plant Biotechnology Journal, 2009. **7**(7): p. 682-693.

Production of Recombinant Protein in Transgenic Barley Grains

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ABSTRACT

Molecular farming is considered a promising strategy to produce valuable proteins not only in human and veterinary medicine, but also in agriculture. Compared with other bioproduction systems such as bacteria, yeasts, mammalian or plant cell cultures, transgenic crop plants may provide an inexpensive and convenient platform for the large scale production of valuable proteins. The particular advantage of barley as a production system is on the one hand that the endosperm provides the foundations to depose a high protein amount and on the other hand that the essential large-scale production processes which include cultivation, harvest, transport and storage are well established. Moreover, outcrossing can be neglected, since barley is a stringent self-pollinator. In this study, we aim to utilise transgenic barley grains to stably express recombinant Anti-F4-scFvBA11 in mature endosperm. Feeding piglets with the milled grains containing these antibodies effectively prevents them from being infected by enterotoxigenic *E. coli* strains. To achieve strong endosperm-specific expression, the *α-Gliadin* promoter of wheat (*Triticum aestivum*) as well as a signal peptide sequence for the targeting of the gene product via the endoplasmic reticulum (ER) to subcellular protein storage compartments is used. Fluorescence analysis of seeds of transgenic barley lines carrying the green fluorescent protein gene (*gfp*) revealed stronger endosperm-specific accumulation of GFP in the ER as compared to cytosolic expression. To further enhance expression and accumulation of recombinant proteins in barley grains we tested 5' untranslated regions (UTR) of the seed-specific legumin *B4* promoter of broad bean (*Vicia faba*) and the 3'UTR of the *Arcelin* (*arc5-I*) gene of common bean (*Phaseolus vulgaris*). Furthermore a combination of the *α-Gliadin* promoter with a fourfold repeat of an enhancer region of the *CaMV35S* promoter was tested to enhance the expression of the *gfp* gene which is utilised as a model in this study. Expectedly, the accumulation and localization of recombinant antibody and GFP in barley caryopses was dependent upon the vector configuration used.

Expression of human acidic fibroblast growth factor in tomato *Solanum lycopersicum*

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In the past decades genetic transformation of plants is a rapidly expanding area with increasing commercial application of the end product. Plants as bioreactors of pharmaceutical proteins are safer in comparison to microorganisms and animal tissues because of the lack of human pathogens, prions, oncogenic DNA sequences and endotoxins. In this work we report the transformation of tomato *Solanum lycopersicum* cv. Bela with the gene for human acidic fibroblast growth factor (haFGF, FGF-1) and phosphomannose isomerase (PMI) as a selectable marker gene.

FGF-1 is a small nonglycosilated molecule of about 16,5 kDa with a cytokine character that binds in a dimeric shape for excretion from the cell. It takes part in the processes of formation and migration of endothelial and smooth muscle cells which are used in therapeutic angiogenesis. The new lymph vessels growth as well as recovery of injured ischemic blood vessels is increased when using such therapy. FGF-1 is used in skin wound and burn healing, pressure ulcer healing etc. PMI (EC 5.3.1.8) is an enzyme that is common in nature, but is less widespread in the plant kingdom. PMI catalyzes the reversible interconversion of mannose-6-phosphate into fructose-6-phosphate. The gene for PMI is an appropriate selectable marker gene for positive selection in plant genetic transformation experiments because plants expressing the PMI gene are able to assimilate the monosaccharide mannose.

Two of the transgenic lines developed via *Agrobacterium*-mediated transformation and their progeny obtained by self-pollination were subjected to analyses for approval of the transgenic nature. PCR, RT-PCR and sequencing of the amplified transgene were performed. For detection of the expression ELISA and Western blot analyses were conducted. Initial experiments concerning allergenicity and toxicity safety assessment of the transgenic plants were carried out. Investigations aiming sequence alignment of the primary structure of haFGF with known allergens from public databases as well as the toxic effect estimation upon laboratory mice were implemented.

Further experiments for biological activity evaluation and optimization of methods for purification of haFGF are planned.

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Aggregation as a bottleneck for IL-10 production in *Nicotiana benthamiana*

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ABSTRACT

For a long time inteleukin-10 (IL-10) has been suggested one of the most promising immunosuppressive molecules. This signaling molecule is mainly used by the immune system to down regulate or prevent immune responses. Produced by monocytes IL-10 inactivates macrophages, disables antigen presentation by dendritic cells and inhibits production of pro-inflammatory cytokines by T cells. But, as with all suggested biopharmaceuticals, its success depends on the ability of the biotechnology industry to produce this molecule cheaply in large quantities. The human IL-10 (hIL-10) gene encodes a 160 amino acid (aa) monomeric protein preceded by an 18aa signal peptide for secretion. The mature protein consists of six alpha helices (A-F) with two internal disulfide bridges. Helices E and F intertwine with helices A-D of another monomer forming a non-covalent homodimer of 37kDa, which is the biologically active form of IL-10. Human and mouse IL-10 (mIL-10) (homology 73%) share a potential glycosylation site which is not glycosylated. mIL-10 has yet another site that is glycosylated, which results in the inability of mIL-10 to trigger a response from human cells. However, hIL-10 can trigger responses from mouse cells. Nowadays, for research purposes IL-10 produced in many different expression systems including *Escherichia coli*, insect cells, Chinese hamster ovarian cells and human cells. Human, mouse and viral IL-10 have also been produced in plants, but accumulation levels are not economically interesting yet. We transiently expressed human and mouse IL-10 in *Nicotiana benthamiana* and found both proteins to be biologically active. Accumulation levels were comparable with levels reported in literature, and the human and mouse proteins could now be compared in the same experimental setup. The difference in accumulation was significant whereby mIL-10 accumulated 28-fold higher then hIL-10. This may be due to the glycosylation event occurring on mIL-10 conferring stability. This implication was supported by the fact that when a tromine-HIS6-KDEL sequence was added accumulation of hIL-10 increased 21 fold and mIL-10 2,3 fold reducing the difference between them to 2,6 fold. Furthermore, as could also be seen on western blots in literature, we found multimeric forms of IL-10 besides the monomeric and biologically active dimeric form. However, none of the previous reports on IL-10 production in plants discusses this phenomenon. We continued to investigate this aggregation and by the use of IL-10-GFP fusions determined it already occurs *in planta* causing golgi-like vesicles to form. By introducing a flexible GS linker between helices D and E, a stable monomeric form of IL-10 was created, previously used for structure-function studies. Expression of this stable monomer in plants increased human IL-10 accumulation 8 fold, suggesting that a crucial limiting factor of accumulation is aggregation by domain swapping.