BEL-VIETNAM R&D cooperation **Project BL/03/V30**

Platforms for plant based production of biopharmaceuticals

Geographic study area: Vietnam

Context and objectives

Plants and plant cells can be used as biofactories and offer many advantages as production systems for biopharmaceuticals: they pose no danger for contamination with human or animal pathogens, they allow production of properly folded and post-translationally modified complex proteins, and they allow cheap and/or fast production of biopharmaceuticals, depending on the exact platform used. Within this project, two plant based production systems are considered: (1) seed-specific expression in stably transformed plants and (2) plant cell cultures transformed with standard vectors or plant-virus-based vectors. On the one hand, improvements of these expression technologies are being developed, and on the other hand these plant-based production platforms are used for the production of veterinary vaccines. In this regard, two major viral diseases are targeted: i.e. avian influenza caused by H5N1 strain and the porcine respiratory and reproductive syndrome caused by PRRSV.

Methodology

WP1: Optimization of vectors for production of an avian influenza vaccine and development of universal vectors for production of plant based biopharmaceuticals
WP2: Expression of PRRSV antigens in *Nicotiana tabacum* cell culture
WP3: Expression of antigens in *Arabidopsis thaliana* and soybean
WP4: Preclinical evaluation of plant produced antigens

Scientific Results

WP1 Broadly applicable vectors for seed specific expression of proteins have been developed. The vectors in particular allow generating transgenic plants free of antibiotic resistance genes or other selectable marker genes. Vector for expression of multiple epitopes of H5N1 virus has been constructed.
The PRRSV GP5 coding sequence was introduced into the binary plasmid pEAQ-HT (Lomonossoff, Norwhich, UK). The plasmid was modified by exchanging the p35S promoter with a home-made strong constitutive promoter (En2pPMA4) or with a heat-inducible promoter that we previously isolated (Navarre et al., Transgenic research, 2011). Construction of vectors with the PRRSV M protein has begun. In addition a novel type of vector for plant cell cultures, based on a plant virus, has been developed. Such vectors might allow very high expression levels in cultured plant cells.
WP2 GP5 transient expression was tested by infiltrating *Nicotiana benthamiana* leaf tissues instead of co-cultivating *N. tabacum* BY2 suspension cells, because it gave better results when we compared the two methods with other proteins, like GFP or HA (Jacquet, 2011). Western blot analysis of transient expression in leaf indicated very low expression of GP5 in the membrane fraction. We then performed a stable transformation of BY-2 cells. Western blot analysis demonstrated no GP5 signal for any clone with the constitutive promoter. This suggests toxicity problems due to GP5 expression. The clones obtained with the inducible promoter are currently being tested.
WP3 H5N1 constructs have been introduced in *Arabidopsis thaliana,* a plant species allowing more rapid evaluation of constructs, and in soybean, a potential large scale production host. Seed from the Arabidopsis transformants were analysed and accumulation levels of H5N1 antigens of more than 5% of total soluble protein were found. Seed produced HA was biochemically active. Accumulation levels of antigens remained the same after long term storage of the seeds at room temperature.
WP4 Arabidospsis seeds producing H5N1 antigens have been used in oral vaccination tests with mice and chicks. In both animals specific immune responses have been detected as verified by ELISA assays and haemagglutination inhibition tests. Oral vaccination tests have also been done with seeds that were stored at room temperature for three years; these experiments showed that the seed expressed antigens remained immunogenic.

Products and services (if applicable: maps, database, peer reviewed article(s),weblink...)

- Website

- International conference proceedings:
*Navarre C., Laterre R., Sallets, Jacquet N. & Boutry M. (2011) Designing transcription promoters for high level expression in plant suspension cells. Plant-Based Vaccines and Antibodies 2011, 8-10 June 2011, Porto, Portugal, Abstract A121
*Angenon G. (2010) Molecular farming: present and future. Conference Proceedings Bio-Hanoi 2010

-(peer review) Journal paper in preparation or published:
Chong-Pérez, B., Kosky, R., Reyes, M., Rojas, L., Ocaña, B., Tejeda, M., Pérez, B. and Angenon G. (2012) Heat shock induced excision of selectable marker genes in transgenic banana by the Cre-lox site-specific recombination system. J**. Biotechnol**. In press
Thu T.T., Son, L.V., Nguyen T.V., Binh, L.T., Angenon G. Oral vaccination with plant seed produced H5N1 antigens, in preparation

---------- Ideas for future research----------
- Plant based production of novel valuable products, specifically from marine organisms
- Forestry biotechnology (improve (Vietnamese) tree species for yield, pathogen resistance, abiotic stress resistance…)

Execution

Period: 2010 – 2013

Laboratory/network:

Belgium: (coordinator and divers partners)
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Discipline

Biotechnology
Veterinary medicine