

## **Dr Tomasz Pniewski**

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### **Scientific specialization**

plant biotechnology; plant transformation – tobacco, lettuce, pea; expression of heterologous protein of bio-pharmaceutical importance

### **Statutory tasks**

Functional analysis of organ-specific legumin A promoter – principal investigator

Analysis of inheritance and activity of transgene controlled by legumin A promoter in progeny generation of transgenic plants - principal investigator

Construction of vectors for plant transformation project of scientific and utilitarian importance - principal investigator

Plant transformation using optimized vectors containing coding sequences of HBV epitopes of increased immunogenicity for an improved anti-HBV vaccine. – principal investigator

Analyses of transgenic tobacco containing coding sequences of HBV epitopes of increased immunogenicity. – principal investigator

### **National co-operation**

1. Institute of Biotechnology and Antibiotics, Warszawa – research on intestinal-mucosal immunization using plant-derived vaccine against hepatitis B
2. Institute of Immunology and Experimental Therapy PAS, Wrocław – research on intestinal-mucosal immunization using plant-derived vaccine against hepatitis B
3. Poznań University of Life Sciences, Department of Microbiology and Food Biotechnology - preparation of plant material for purposes of an oral vaccine against hepatitis B.
4. Adam Mickiewicz University, Department of Biology, Poznań – detection of Virus-Like Particles assembled by HBV antigens in plant cells
5. Medana S.A., Sieradz – exploitation of transgenic plants for production of vaccines against hepatitis B.
6. Institute of Plant Breeding and Acclimatization, Laboratory of Oilseed Crops, Poznań – Elaboration of transformation method of winter oilseed rape, transformation of winter oilseed rape for scientific and utilitarian purposes

### **International co-operation**

1. Institut Pasteur, Paris, France, dr Monica Sala – exploitation of transgenic plants for preparation of a bivalent vaccine against HIV-1 and HBV

### **Projects**

Research Project of Polish Ministry of Science and Higher Education, No. N N302 157837, „Mucosal-intestinal immunization of mice as model animals using prepared plant material containing S-HBsAg for purposes of an oral vaccine against hepatitis B.” realized in co-operation of IPG PAS Poznań with IIET PAS Wrocław, ULS Poznań and IBA Warsaw, 2009-2012, principal investigator

Research Project of Polish Ministry of Science and Higher Education, No. PBZ/MNiSW/07/2006/15 „Purification and immunogenicity analysis of virus-like particles

assembled of plant-expressed HBV antigens as components of III generation vaccine against hepatitis B.” realized in co-operation between IPG, Poznań and IBA, Warsaw, 2007-2010, investigator

Project of Polish Ministry of Science and Higher Education within Patent Plus programme, No. PP/008/DWI/2007 “International patent of the invention „Expression cassette, T-DNA molecule, plant expression vector, transgenic plant cell as well as their use in the manufacturing of a vaccine” related to plant-derived vaccine against HBV”, 2007-2009, coordinator

Research Project of Polish Ministry of Science and Information Infrastructure, No. 2 P04B 001 27 „Expression of HBV antigens in transgenic plants for the recombinant new generation vaccine against hepatitis B.”, 2004 – 2007, principal investigator

#### **Patents awarded:**

- The chimeric protein, sequence, construct, plant cell, the method of obtaining chimeric protein and transgenic plants, transgenic plant, the application of the transgenic plant and chimeric protein – Classic Swine Fever Virus (CSFV)  
No. P 357518, published 14.06.2004  
Legocki AB, Kapusta J, Pniewski T, Lisowa O, Miedzinska K, Czaplńska M, Femiak I, Płucienniczak A, Kęsik M, Porębska A, Szewczyk B, Gut-Winiarska M, Tyborowska J, Ficińska J, Bieńkowska–Szewczyk K, Wojciechowicz J
- The chimeric protein, sequence, construct, plant cell, the method of obtaining chimeric protein and transgenic plants, transgenic plant, the application of the transgenic plant and chimeric protein – fascioliosis (*Fasciola hepatica* L.).  
No. P 357517, published 14.06.2004  
Legocki AB, Miedzinska K, Czaplńska M, Modelska A, Pniewski T, Płucienniczak A, Kęsik M, Porębska A, Wędrychowicz H, Mieszczanek J, Jedlinek-Panasiuk L

#### **Patents applied**

- “Expression cassettes, T-DNA fragments, plant expression vectors, transgenic plant cells and their application for making of the vaccine.”, International Patent Application No. PCT/PL2010/050003, applied 2010, Pniewski T, Kapusta J, Płucienniczak A, Płucienniczak G, Bociąg P, Kostrzak A, Fedorowicz-Strońska O, Krajewski P, Wolko B
- “Expression cassette, T-DNA fragment, plant expression vector, transgenic plant cell and their application for making of the vaccine.” Nr PCT/PL2008/000046 , patent application in European Patent Office and US Patent and Trademark Office, applied 2010, Pniewski T, Kapusta J, Płucienniczak A, Płucienniczak G, Wojciechowicz J, Bociąg P, Wójcik P, Otta H, Kostrzak A, Wolko B
- Expression cassettes, T-DNA molecules, plant expression vectors, transgenic plant cells as well as their use in the manufacturing of a vaccine, No. 387114, national application 2009, Pniewski T, Kapusta J, Płucienniczak A, Płucienniczak G, Bociąg P, Fedorowicz-Strońska O, Kostrzak A, Krajewski P, Wolko B
- Expression cassette, T-DNA molecule, plant expression vector, transgenic plant cell as well as their use in the manufacturing of a vaccine, No. P382769, national application

2007, Pniewski T, Kapusta J, Płucienniczak A, Płucienniczak G, Wojciechowicz J, Bociąg P, Wójcik P, Otta H, Kostrzak A, Wolko B

- Chimaeric protein containing cysteine protease of liver fluke fused to hepatitis B core protein or ubiquitin, plants expressing said protein and uses thereof as vaccine. No. 03786456.8-2406-PL0300135, applied 2003, Legocki AB, Miedzinska K, Czaplńska M, Modelska A, Pniewski T, Płucienniczak A, Kęsik M, Porębska A, Wędrychowicz H, Mieszczanek J, Jedlinek-Panasiuk L

## Publications

1. Pniewski T, Kapusta J, Bociąg P, Wojciechowicz J, Kostrzak A, Gdula M, Fedorowicz-Strońska O, Wójcik P, Otta H, Samardakiewicz S, Wolko B, Płucienniczak A (2011) Low-dose oral immunization with lyophilized tissue of herbicide-resistant lettuce expressing hepatitis B surface antigen for prototype plant-derived vaccine tablet formulation. *J App Genet* 52: 125-136.
2. Kapusta J, Pniewski T, Wojciechowicz J, Bociąg P, Płucienniczak A (2010) Nanogram doses of alum-adsorbed HBs antigen induces humoral immune response in mice when orally administered. *Arch Immunol Ther Exp* 58:143–151.
3. Kostrzak A, Cervantes Gonzalez M, Guetard D, Nagaraju DB, Wain-Hobson S, Tepfer D, Pniewski T, Sala M (2009) Oral administration of low doses of plant-based HBsAg induced antigen-specific IgAs and IgGs in mice, without increasing levels of regulatory T cells. *Vaccine* (27):4798-4807.
4. Gonzalez MC, Kostrzak A, Guetard D, Pniewski T, Sala M (2009) HIV-1 derived peptides fused to HBsAg affect its immunogenicity. *Virus Res.* 146:107-114.
5. Cegielska-Taras T, Pniewski T, Szala L (2008) Transformation of microspore-derived embryos of winter oilseed rape (*Brassica napus* L.) using *Agrobacterium tumefaciens*. *J Appl Genet* 49: 343-347.
6. Pniewski T (2007) Expression of S-HBs antigen in transgenic plants for developing a recombinant vaccine against hepatitis B. Annual Report 2007 Polish Academy of Sciences, 80-83.
7. Cegielska-Taras T, Pniewski T, Szala L (2007) Transformation of microspore-derived embryos of winter oilseed rape (*Brassica napus* L.) using *Agrobacterium tumefaciens*. 12<sup>th</sup> International Rapeseed Congress, Wuhan, China, 26-30. 03.2007, str. 24 - 26.
8. Kapusta J, Pniewski T (2006) „Biotechnologia w medycynie” w: „Biologia molekularna w medycynie. Elementy genetyki klinicznej” Red. Jerzy Bal, Warszawa PWN, str. 550-584.
9. Pniewski T, Kapusta J, Płucienniczak A (2006) “*Agrobacterium tumefaciens*-mediated transformation of yellow lupin to generate callus tissue producing surface antigen of HBV in a long-term culture. *J Appl Genet* 47: 309-318.
10. Pniewski T, Kapusta J (2005) “Efficiency of transformation of Polish cultivars of pea (*Pisum sativum* L.) with various regeneration capacity by using hypervirulent *Agrobacterium tumefaciens* strains.” *J Appl Genet* 46: 139 – 147
11. Pniewski T, Wachowiak J, Kapusta J, Legocki AB (2003) Organogenesis and long-term micropropagation of Polish pea cultivars. *Acta Soc Bot Pol* 72: 295-302.
12. Pniewski T, Kapusta J, Legocki AB (2002) *In vitro* micropropagation of four lupin species. *Acta Physiolo Plant* 4: 417-424.

13. Cegielska-Taras T, Pniewski T, Szala L, Miedzinska K (2002) Gene transfer by *Agrobacterium tumefaciens* to microspores and microspore-derived embryos of winter oilseed rape (*Brassica napus* L.). Bulletin GCIRC 2002, publication(B18)contents.htm
14. Kapusta J, Modelska A, Pniewski T, Figlerowicz M, Jankowski K, Lisowa O, Plucienniczak A, Koprowski H, Legocki AB (2001) Oral immunization of human with transgenic lettuce expressing hepatitis B surface antigen. *Adv Exp Med Biol.* 495: 299-303
15. Kapusta J, Modelska A, Figlerowicz ., Pniewski T, Letellier M, Lisowa O, Yusibov V, Koprowski H, Płucienniczak A, Legocki AB (1999) A plant-derived edible vaccine against hepatitis B virus. *FASEB J.* 13: 1796 – 1799.

### **Research and practical achievements:**

1. Expression of HBV antigens in transgenic plants. Transgenic herbicide-resistant lettuce and tobacco plant were obtained expressing particular proteins of HBsAg at a level reaching several tens of  $\mu\text{g/g}$  FW and HBcAg at a level up to 200  $\mu\text{g/g}$  FW.
2. Expression of HIV1-HBV antigens in transgenic plants for a potential bivalent vaccine against AIDS and HBV. Transgenic tobacco was obtained expressing two-component particular Virus-Like Particles assembled of HIV1 polyepitope-HBsAg and S-HBsAg at a level up to several tens  $\mu\text{g/g}$  FW.
3. Pilot oral (intestinal-mucosal) immunization of mice and humans using a plant-derived prototype of oral vaccine containing HBV antigens. Induction of specific anti-HBs antibodies in intestinal mucosa and serum was demonstrated.
4. Development of micropropagation method of transgenic lettuce expressing HBV antigens HBV making possible a mass propagation of plant material characterizing stable and uniform level of HBV antigens for purposes of vaccine preparation.
5. Development of ELISA test for assay of HBV antigens in plant material.
6. Development of the tablet-form plant-derived prototype vaccine against HBV.
7. Elaboration of regeneration and transformation protocols of lettuce, pea and lupin.