## Molecular Farming: Plants Producing Vaccine and Diagnostic Reagent

Authors: Katerina H. Takova, Ivan N. Minkov, Gergana G. Zahmanova

Abstract: Molecular farming is the production of recombinant proteins in plants with the aim to use the protein as a purified product, crude extract or directly in the planta. Plants gain more attention as expression systems compared to other ones due to the cost effective production of pharmaceutically important proteins, appropriate post-translational modifications, assembly of complex proteins, absence of human pathogens to name a few. In addition, transient expression in plant leaves enables production of recombinant proteins within few weeks. Hepatitis E virus (HEV) is a causative agent of acute hepatitis. HEV causes epidemics in developing countries and is primarily transmitted through the fecal-oral route. Presently, all efforts for development of Hepatitis E vaccine are focused on the Open Read Frame 2 (ORF2) capsid protein as it contains epitopes that can induce neutralizing antibodies. For our purpose, we used the CMPV-based vector-pEAQ-HT for transient expression of HEV ORF2 in Nicotiana benthamina. Different molecular analysis (Western blot and ELISA) showed that HEV ORF2 capsid protein was expressed in plant tissue in high-yield up to 1g/kg of fresh leaf tissue. Electron microscopy showed that the capsid protein spontaneously assembled in low abundance virus-like particles (VLPs), which are highly immunogenic structures and suitable for vaccine development. The expressed protein was recognized by both human and swine HEV positive sera and can be used as a diagnostic reagent for the detection of HEV infection. Production of HEV capsid protein in plants is a promising technology for further HEV vaccine investigations. Here, we reported for a rapid high-yield transient expression of a recombinant protein in plants suitable for vaccine production as well as a diagnostic reagent. Acknowledgments -The authors' research on HEV is supported with grants from the Project PlantaSYST under the Widening Program, H2020 as well as under the UK Biotechnological and Biological Sciences Research Council (BBSRC) Institute Strategic Programme Grant 'Understanding and Exploiting Plant and Microbial Secondary Metabolism' (BB/J004596/1). The authors want to thank Prof. George Lomonossoff (JIC, Norwich, UK) for his contribution.

**Keywords:** hepatitis E virus, plant molecular farming, transient expression, vaccines

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