

## Expression behaviour of the fusion antigen CTB::VP60, a promising RHD-vaccine, in pea

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The production of veterinary vaccines in transgenic plants appears to be an upcoming strategy. Since 15 years transgenic plants express a number of viral and bacterial antigens -amongst others VP60 (1, 2), the only structural capsid protein of the rabbit haemorrhagic disease virus (RHDV). However plant-derived VP60 demonstrated very low immunogenicity compared to other production systems like baculovirus.

In order to enhance the immunogenicity of plant-derived VP60 we fused the protein to the well known adjuvant CTB, the non-toxic B subunit of Cholera toxin (CTA-CTB<sub>5</sub>). The chimeric gene *ctbvp60SEKDEL*, regulated by the CaMV 35S promoter, was integrated into pea (*Pisum sativum*) by *Agrobacterium*-mediated transformation. Around 60 independent transgenic plant lines of the F1 generation were analysed and the protein content of CTB::VP60 was estimated using CTB-, GM1-ganglioside- and VP60-dependent ELISA. Four transgenic F1 lines demonstrated detectable amount of the two complex components CTB and VP60. The heterogeneous pentamer complex could be detected by a newly developed combined GM1-VP60-ELISA Test.

Additionally, we attempted to increase expression levels by analysing transgenic plant lines in the F2 and F3 generation after self pollination of the two highest expression lines. Up to 100 independent transgenic F3-lines with significant enhancement of the expression level up to 0.7 % TSP were selected to produce CTB::VP60-seeds for immunisation experiments with rabbits using mucosal and parenteral vaccination strategies.

### References

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