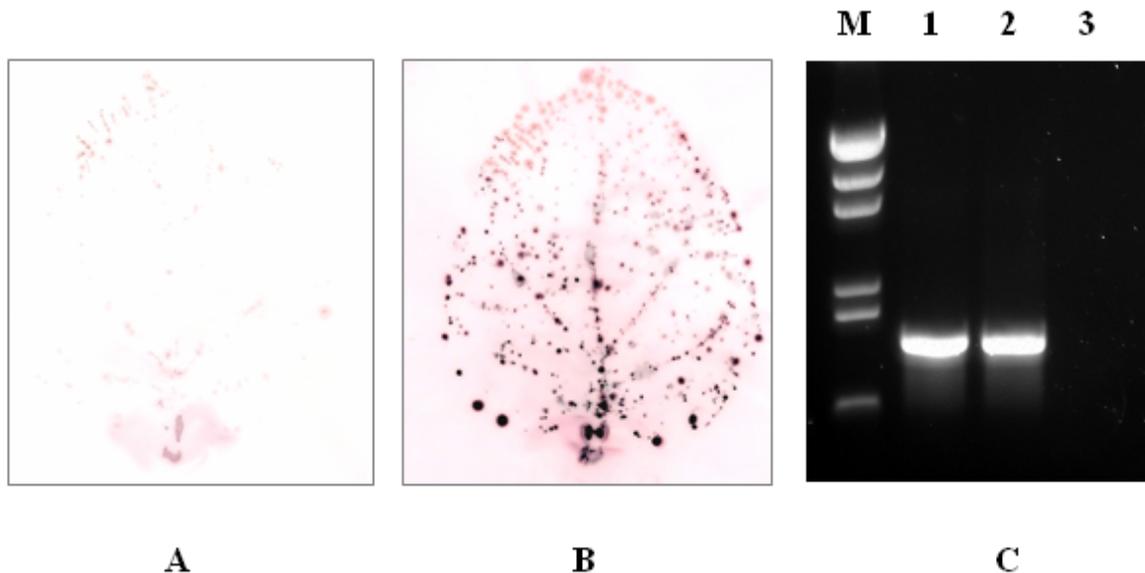


## Transient expression of the ectodomain of matrix protein 2 (M2e) of avian influenza A virus in plants

We have previously reported an expression system based on the capsid protein gene (CP) of *Cucumber mosaic virus* (CMV) placed under transcriptional control of a *Potato virus X* (PVX)-based vector. PVX-expressed CMV CP formed virus-like particles, which served as carriers for heterologous antigens of the *Newcastle disease virus* (NDV).

In this work, we applied our expression tool toward the development of plant-derived vaccine candidate against avian influenza A virus. The twenty three amino acid-long extracellular domain of the viral M2 protein (M2e) was engineered into the internal motif 5 of CMV CP and the recombinant gene then was transiently expressed in plants through a PVX vector. Chimeric CMV capsids reacted with specific antibodies produced to synthetic M2e epitope of the H5N1 strain of the virus. In addition, CMV CP-M2e protein was expressed to high levels in *Escherichia coli* bacterial cells and was recognized by antibodies to both CMV and M2e. This initial study demonstrates the feasibility of using plant virus-based vectors for expression of antigenic epitopes of H5N1 avian influenza in plants.



**A** and **B**, whole leaf squash blots probed with antibodies to the M2e epitope. **A**, plant infected with recombinant PVX containing a wild type gene of CMV-Ix coat protein; **B**, plant infected with PVX containing the CMV CP-M2e fusion. **C**, RT-PCR products amplified from plants infected with recombinant PVX/CMV CP- M2e virus using CMV CP-specific primers. **M**, Lambda DNA *EcoRI/HindIII* marker (Promega): 2, 1.5, 1.3, 0.9, 0.8 and 0.5 kb. **Lane 1 and 2**, RT-PCRs from two different plants infected with PVX/CMVCP-M2e virus. **Lane 3**, RT-PCR reaction from plant infected with wild type PVX.

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