

SARS-CoV NUCLEOCAPSID AND MEMBRANE PROTEINS EXPRESSED IN PLANTS FOR THE DEVELOPMENT OF LOW COST DIAGNOSIS TEST AND ORAL VACCINE.

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The *Coronavirus* SARS-CoV is the aetiological agent of the Severe Acute Respiratory Syndrome (SARS), which caused a worldwide infectious emergence in 2003. There is no specific therapy or vaccine against this diseases, thus we have started investigations in this area as well as for the generation of low cost diagnostic tests. Working with SARS-CoV is potentially hazardous, as the virus has a recognised ability to infect laboratory workers, and there is a need to provide a safe way to produce SARS antigens that can be manufactured with minimal risk.

The SARS-CoV M and N genes (encoding for the most abundant proteins, respectively, in the virus core and in the viral envelope) were previously reconstituted by RT-PCR on viral RNA extracted by the SARS-CoV Frankfurt isolate and were expressed in *E. coli* to develop a serological assay for diagnosis of SARS. As the integral M protein is toxic in *E. coli* and N protein is mainly expressed in inclusion bodies, we decided to express these proteins in *Nicotiana benthamiana* plants by using a Potato Virus X (PVX)-derived vector, to try to get these proteins in native form. Both proteins were obtained in systemic leaves after PVX infection.

In particular, the N protein was expressed as soluble (about 3 µg recombinant protein/g of fresh plant tissue) and was specifically recognized by rabbit and mouse hyperimmune sera that also recognize the protein in SARS-CoV infected cells. With the aim to evaluate the immunogenicity of the plant-derived antigens and to develop a diagnostic test based on plant extracts containing the SARS-CoV N protein, we are evaluating the reactivity with the plant extracts of sera from patients with confirmed SARS diagnosis.

The results might open the way for the development of low-cost plant-derived SARS-CoV diagnostic tools. Moreover, we are pursuing this approach for the generation of (edible) vaccines against SARS.