



## Expressing Hepatitis B Virus Surface Antigen in Rice

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Hepatitis B virus, which infects the liver of hominidae, including humans, is one of many unrelated viruses that cause viral hepatitis. The proportion of the world's population currently infected with the virus is estimated at 3% to 6%. Chronic hepatitis B infection may eventually cause liver cirrhosis and liver cancer, a fatal disease with a very poor response to current chemotherapy. The disease was originally known as 'Serum Hepatitis' and has caused epidemics in parts of Asia and Africa, and is endemic in China and various other parts of Asia. The infection is preventable by vaccination, and after the development of a commercial recombinant vaccine derived from yeast, there was a decline in the spread of HBV infection. Because plants may be promising bioreactors for producing vaccines, much research is geared toward the cost effective production of recombinant surface antigens in plants. For example, researchers from Fudan University, Jiao Tong University, and the Institute for Biological Sciences, Shanghai, China recently reported producing a novel hepatitis B vaccine in rice seeds.

The team constructed a rice endosperm-specific vector containing the *SSI* gene, which expresses a modified hepatitis B virus (HBV) surface antigen (HBsAg). To do this, a 2.8 kb *SSI* expression cassette was cloned into p<sup>CAMBIA</sup>1300 to produce the p1300GSS1 plant binary vector. Transgenic rice plants (*Oryza sativa* L.) were produced through *Agrobacterium* mediated transformation. Incorporation of the genome into the target gene was confirmed by PCR and Southern blot analysis. Also, RNA dot blot analyses were performed to further test whether the fused *SSI* gene was specifically expressed in rice seeds. The amount of recombinant SS1 protein in transgenic rice seeds was measured by quantitative ELISA. A CsCl gradient analysis was performed to find out whether the introduced recombinant SS1 in rice plants formed virus-like particle (VLP) structures. To observe VLPs in the recombinant SS1 protein fraction, solid phase immune electron microscopy was used.

Finally, the immunogenic response of the recombinant SS1 protein was tested in adult BALB/c female mice. In this experiment, mice aged 6 to 8 weeks were immunized intraperitoneally three times at two-week intervals with the freeze-dried total protein of all the transgenic rice seeds. Each dose contained 0.5 µg of recombinant SS1 protein emulsified with 200 µl of complete Freund's adjuvant in a final volume of 400 µl at the first immunization, and with incomplete Freund's adjuvant at subsequent immunizations. A control experiment was also carried out with another group of eight adult mice, immunizing them with the total protein of non-transgenic rice seeds. Because the aim of the research was to generate recombinant protein with S (HBV surface protein) and preS1 (presurface 1 region) epitope immunogenicity, the presence of antibodies against S and preS1 in mice sera was tested using indirect ELISA.

The study concluded that SS1 was successfully expressed in the rice plants. Of 416 regenerated plants, 164 were transgenic and exhibited normal growth in field conditions when compared to non-transgenic rice plants. One plant exhibited a high expression level and accumulated the highest amount of recombinant SS1 protein, at  $\sim 31.5 \pm 1.4$  ng/g dry weight (DW) grain. Others showed slightly lower expression levels, ranging from  $15.8 \pm 0.7$  to  $26.3 \pm 1.1$  ng/g DW grain, and five plants showed no expression.

Further Western blot analysis using antibodies against S and preS1 protein indicated that the rice-derived recombinant SS1 protein possessed both S and preS1 antigenicity. The results also confirmed that the recombinant SS1 protein could self-assemble into VLPs, which is indicative of a strong immunogenic response. Recombinant SS1 from transgenic rice induced a specific antibody response against both S and preS1 in immunized mice. Specific preS1 antibodies, which were detected four weeks after the initial antigen reaction, reached a peak value of  $0.475 \pm 0.055$  (OD<sub>450:630</sub>) three weeks after the final boosting injection. Specific S antibodies were first detected five weeks after the initial antigen injection, and reached a peak of  $0.446 \pm 0.053$  (OD<sub>450:630</sub>) in the eighth week. During subsequent weeks antibodies against both S and preS1 remained at relatively high levels. The control mice immunized with crude proteins from non-transgenic rice seeds showed no immune response to S and preS1.

In summary, the researchers expressed recombinant SS1 protein in rice seeds, which in turn induced immunological responses against S and preS1 protein in mice. From this study we can infer that the rice-derived SS1 protein could be developed as an alternative oral vaccine for preventing HBV infection in humans.

### Source

Qian B *et al.* (2007) Immunogenicity of recombinant hepatitis B virus surface antigen fused with preS1 epitopes expressed in rice seeds. *Transgenic Research* DOI 10.1007/s11248-007-9135-6 (Online First edition)

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