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ORGAN-SPECIFIC EXPRESSION OF HEPATITIS B SURFACE ANTIGEN IN POTATO.

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SUMMARY

The gene encoding the hepatitis B surface antigen (HBsAg) under the control of cauliflower mosaic virus 35S-promoter was constructed and expressed in transgenic potato plants. The HBsAg expression were measured by ELISA kit containing monoclonal antibodies. The amount of HBsAg in roots was found 5-10 fold higher than in leaf tissues

INTRODUCTION

Hepatitis B virus infection remains an important public health problem all over the world and especially in many developing countries. As immunization represents the only known method to prevent the spread of the virus , any concerted attempt to reduce the incidence of hepatitis B infection necessitates the availability of large quantities of suitable vaccine antigen. To date, the production of hepatitis surface antigen using recombinant DNA techniques is the most promising and effective way (Murray, 1988). However, despite the safety and efficacy of the genetically engineered vaccines the high costs associated with mass vaccination have led to the search for alternative methods of producing HBsAg for effective hepatitis B vaccines (Higashihashi et al., 1991; Janowicz et al., 1991; Mason et al., 1992; Schodel et al., 1994).

Recently, it was shown that HBsAg which is recognized specifically by monoclonal antibodies can be expressed in tobacco plant tissues via stable transformation with the recombinant plasmid DNA (Mason et al., 1992). These data opened the very attractive possibility to use the transgenic plants expressing foreign genes as oral vaccine carriers.

In this paper we describe the transformation of potato with the gene encoding HBsAg and its expression in transgenic plants.

MATERIALS AND METHODS

The coding sequence for HBsAg subtype *ayw* (Bichko et al., 1985) was excised from the pMT-HBsAg plasmid (kindly provided by Prof. K. G. Gazaryan from Russian Academy of Sciences) as a BglII - BamHI fragment and cloned into the pCaMV plasmid containing a unique BamHI site between CaMV 35S-promoter and polyadenylation site of the nopaline synthase gene (Fromm et al., 1986). The constructed chimeric gene was then released with HindIII and inserted into a binary vector Bin19 (Bevan, 1984). *Agrobacterium* strain pGV3850 cells were transformed by direct method (Burow et al., 1990) with the plasmid prepared from *E. coli* clone and structure of plasmids was verified by restriction digestion and PCR analysis. Potato (*Solanum tuberosum* cv. Nevsky) was transformed by cocultivating *in vitro*-grown minituber discs with *Agrobacterium* strain (Ishida et al., 1989). HBsAg was isolated from plant tissues by differential centrifugation (Mason et al., 1992). The extractable tissue HBsAg was measured by an ELISA kit (Hepadiagnostika® HBsAg, Organon Teknika B.V., Holland) and quantitative HBsAg levels were determined by serial dilutions and comparison with a standard curve with the use of purified HBsAg of known concentration. Total soluble proteins were determined according to Lowry (Lowry, 1951).

RESULTS AND DISCUSSION

Construction of the chimeric gene is described in Materials and Methods and the structure is schematically shown in Fig. 1. According to the information found in EMBL genetic sequence data bank (accession N X02496), the inserted DNA fragment of HBV subtype *ayw* contains a sole open reading frame with the unique initiation codon for HBsAg. Thus the recombinant protein can be encoded only by this open reading frame.

Constructed chimeric gene was recloned into Bin19, mobilized into *Agrobacterium* and used for transformation of potato minitubers. A total of 5 kanamycin-resistant shoots were selected and *in vitro*-grown plants were regenerated.

The regenerated plants were assayed for the HBsAg expression using the commercially available Hepadiagnostika® HBsAg system. Whole plants were used for the initial analysis and the OD readings that exceeded the cut-off level more than twice were obtained.

These data suggest that the HBsAg expressed in transgenic potato plants is recognized specifically by monoclonal antibodies directed against human serum-derived HBsAg. The reaction was specific because non-transgenic plants showed no detectable HBsAg.

According to our estimates, the concentration of HBsAg in the protein extracts from plant tissues varied from 3 to 11 ng per mg of soluble proteins in leaves and from 76 to 83 ng per mg in roots of transgenic plants. Approximately the same level of the HBsAg expression in tobacco leaves has previously been reported (Mason et al., 1992).

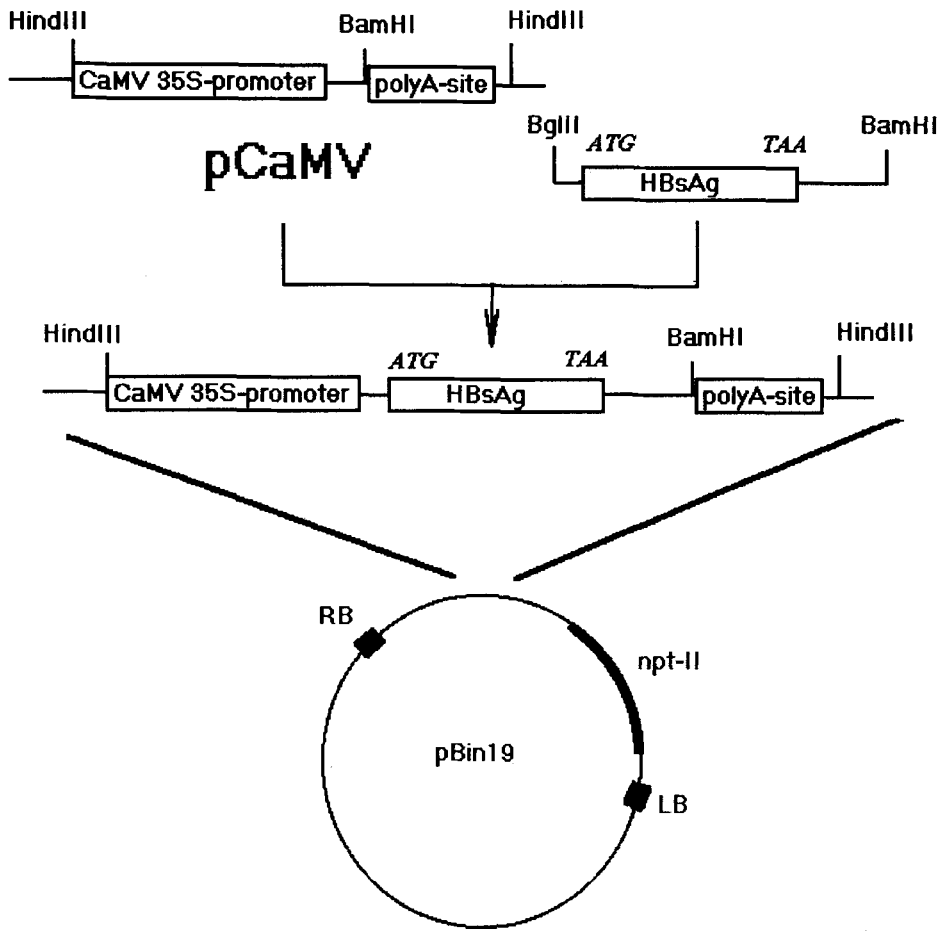

Fig. 1. A shematic representation of plasmid construction for the expression of HBsAg in plants.

Abbreviations are: RB, LB- left and right border sequences of Ti-plasmid; npt-II - kanamycin-resistance marker for use in plant cells.

Our results suggest that the HBsAg expression is higher in roots of the transgenic plants that in agreement with the data that the concentration of the activation sequence factor ASF-1 which activates the 35S-promoter is 5 to 10 fold higher in roots than in leaf tissue (Benfey et al., 1990).

Of course, the maximal level of the HBsAg expression we have obtained in transgenic potato plants is not sufficient for the use of these plants for production of the HBsAg vaccine, as the expression of HBsAg in yeast system reaches 250 μg per 400 mg of soluble protein (Hartford et al., 1982). However, it must be emphasized that the optimal level of the HBsAg expression in plant tissues for their use as an oral vaccine still remains to be experimentally determined.

To increase the level of the HBsAg expression in potato plants by using patatin promoter and transcription regulatory element of tobacco etch virus further studies are now under way.

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