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Research article

Expression of Hemagglutinin–Neuraminidase and fusion epitopes of Newcastle Disease Virus in transgenic tobacco

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ABSTRACT

Background: Newcastle disease is an important avian infectious disease that brings about vast economic damage for poultry industry. Transgenic plants represent a cost-effective system for the production of therapeutic proteins and are widely used for the production of poultry vaccines. In an attempt to develop a recombinant vaccine, a plant expression binary vector pBI121, containing the genes encoding Hemagglutinin–Neuraminidase (HN) and Fusion (F) epitopes of Newcastle Disease Virus (NDV) under the control of CaMV35S promoter and NOS terminator was constructed and introduced into the tobacco (*Nicotiana tabacum*) plant by *Agrobacterium*-mediated transformation. *Results:* Putative transgenic plants were screened in a selection medium containing 50 mg/L kanamycin and 30 mg/L meropenem. Integration of the foreign gene in plant genome was confirmed by PCR. Expression of foreign gene was analyzed at transcription level by RT-PCR and at translation level by means of dot blotting and ELISA. All analyses confirmed the expression of recombinant protein.
Conclusion: Developments in genetic engineering have led to plant-based systems for recombinant vaccine production. In this research, tobacco plant was used to express F and HN epitopes of NDV. Our results indicate that for the production of recombinant vaccine, it is a novel strategy to use concatenated epitopes without their genetic fusion onto larger scaffold structure such as viral coat protein.

1. Introduction

Newcastle Disease Virus (NDV) is an economically important pathogen that infects both wild and domesticated birds [1,2]. NDV belongs to the *Rubulavirus* genus and Paramyxoviridae family and is a negative-sense, single-stranded RNA virus with 15 kb genome. The genome encodes six major structural and non-structural proteins including nucleocapsid (NP), phosphoprotein (P), matrix (M), fusion (F), Hemagglutinin–Neuraminidase (HN) and RNA-dependent RNA polymerase (L) [3]. F and HN are glycoproteins that are critical for virulence and these two surface proteins are the most important targets for the host immune response and induce neutralizing antibody against NDV [3]. Amino acids 65–81 of F protein and 346–353 of HN have been identified as the most important immunogenic sites for antibody induction [4]. Killed or attenuated viruses are currently used as anti–NDV vaccine [4]. Although these vaccines are effective, high cost of vaccination, side effects such as egg decrease in chickens, high labor cost and stress that may lead to a reduction in egg-laying, or to an increased susceptibility to microorganisms infections call for a new method of production of NDV vaccines [4]. The best route of vaccination against NDV is oral administration as vaccines can be incorporated in poultry diet [5]. Production of recombinant vaccines based on capsid subunits and their application as oral vaccines is an effective alternative for conventional attenuated virus-based vaccines [6].

Plants represent an ideal platform for the production of recombinant vaccines [7]. Transgenic plants expressing foreign proteins of industrial and therapeutic value are good alternatives for fermentation systems. Various vaccines expressed transiently or permanently in green plants showed accurate conformation for the induction of protective and neutralizing immune responses in human, animal and poultry [7]. A major advantage of plant-based recombinant vaccines — in addition to ease of production and administration — is the induction of mucosal immunity which subsequently results in high immunity for the host. Considering that oral or nasal vaccine — delivery is more effective at

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stimulating mucosal immunity, it would be quite fitting to express antigens in plants to be delivered as edible vaccines [7]. The successful use of transgenic plants for the expression of a number of pathogen antigens has revealed new prospects for vaccine production [8,9].

Due to numerous advantages, tobacco is widely used in genetic transformation as a plant host. Advantages include ease of transformation, availability of optimized tissue culture systems and high level of transgene expression [10]. Thus, this plant species is commonly used in scientific research as a plant host and when successful, the gene of interest can be transferred into other plants [10,11].

The present study aims to transfer and express F and HN epitopes in tobacco. Since tobacco is an ideal model plant for transgene expression, the successful expression of the epitopes in this plant can be the beginning for the commercial production of recombinant anti-NDV vaccines in other plant species.

2. Materials and methods

2.1. Designing gene construct

Four tandem repeats of HN epitope (encoding amino acids 346–353) with 96 bp length followed by three tandem repeats of F epitope (encoding amino acids 65–81) with 153 bp length were used. The sequences were retrieved from NCBI. The gene was codon optimized to ensure high level of expression. The initiation codon (AUG) followed by histidine tag (18 bp) and omega sequence as ribosome binding (67 bp) site were attached in upstream of the gene. The endoplasmic reticulum signal (SEKDEL) was included to 3′ end just before termination codon. Finally, two restriction sites namely *BamHI* and *SacI* were added to 5′ and 3′ ends. The final length of the gene was 376 bp. Gene was designed by CLC software (Fig. 1). The resulting gene construct was synthesized and cloned in pGH vector by Gene ray Company.

2.2. Construction of pBI121 construct containing F and HN antigens

Gene construct was removed from pGH via digestion by *BamHI* and *SacI*; binary vector pBI121 containing CaMV 35S promoter and NOS terminator was digested by the same enzymes and after removing GUS sequence, gene construct of F and HN epitopes was inserted between promoter and terminator (Fig. 2). The resulting construct is called pBI121-NDV epitopes which was transferred to agrobacterium using electroporation. To confirm the presence of the plasmid within bacterial cell after extraction and digestion by *HindIII*, a fragment of about 1096 bp was revealed on electrophoresis gel (Fig. 3).

2.3. Agrobacterium-mediated transformation of tobacco

Agrobacterium tumefaciens strain C58 harboring pBI121-NDV epitopes was cultured in LB medium for 18 h. The bacterial suspension at $OD_{600} = 0.6$ was centrifuged at 3500 × g for 20 min. Bacterial pellet was cultured for 120 min at 28°C in transformation medium (MS salts, 5% glucose and 200 μM acetosyringone, pH = 7.5).

Leaf disc method was applied to transform tobacco plant. Briefly, surface sterilized leaf discs of *Nicotiana tabacum* cv. Turkish were co-cultivated with *A. tumefaciens* suspension carrying pBI121-NDV epitopes plasmid for 15 min, then transferred into MS medium containing 2 mg/L BA and 2 mg/L NAA and incubated in darkness. After 48 h, the discs were washed with 30 mg/L meropenem to eliminate agrobacterium. The explants were then transferred to regeneration medium containing MS salts, 2 mg/L BA, 2 mg/L NAA, 50 mg/L kanamycin and 30 mg/L meropenem at 25°C and photoperiod of 16 h light/8 h darkness. Subculture was done every two weeks and for rooting of the explants, the regenerated plantlets were cultured in root induction medium (MS containing 2 mg/L IBA).

2.4. DNA extraction and PCR

To determine the presence of foreign gene in genome of the regenerated seedlings resistant to kanamycin, genomic DNA was extracted from young leaves of the seedlings and from wild type plants using the method proposed by Dellaporta et al. [12]. PCR was performed by specific primers with sequences of 5′TCATTGCGATAAAG GAAAGGC3′ and 5′AATGTATAATTGCGGGACTC3′. PCR was carried out by 35 cycles of 94°C for 60 s, 54°C for 60 s and 72°C for 60 s, followed by a final extension step of 72°C for 10 min. DNAs of wild type plant and plasmid pBI121-NDV epitopes were used as negative and positive control; respectively. PCR product was segregated on 1% agarose gel.

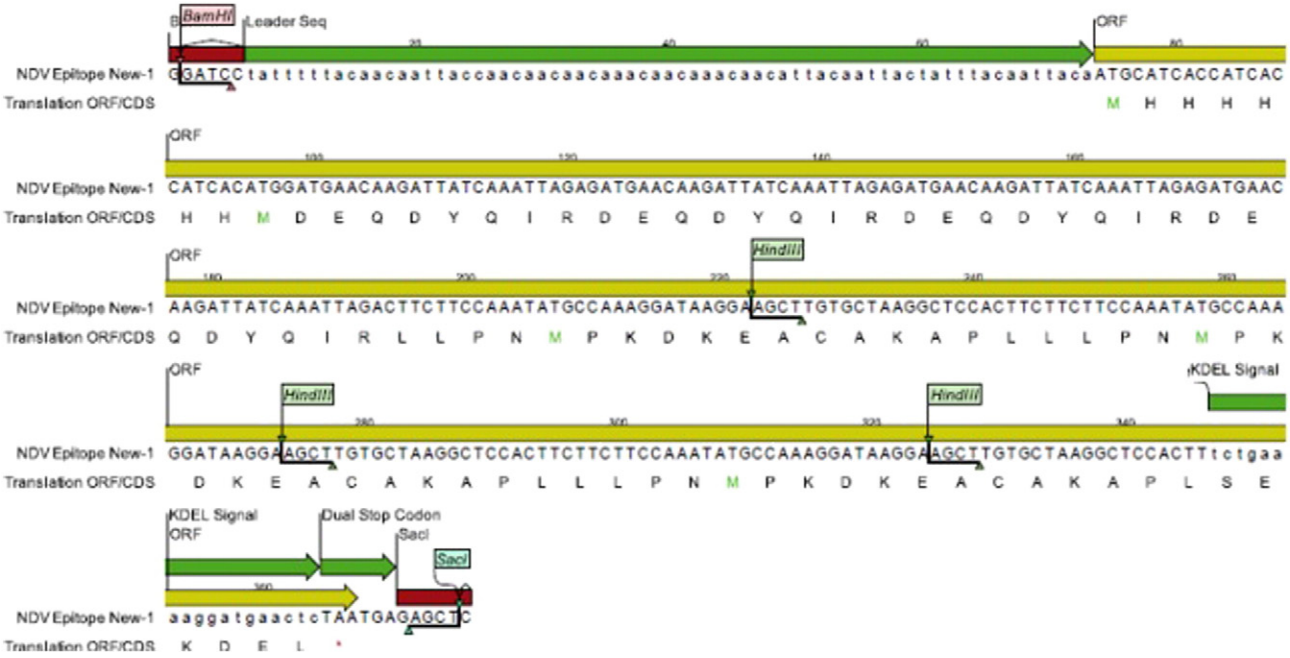

Fig. 1. Gene construct including F and HN epitopes together with needed sequences for expression in tobacco.