The Use of Transgenic Tobacco as a Production and Delivery System for a Vaccine Against Hemorrhagic Enteritis Virus of Turkeys

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(ABSTRACT)

Hemorrhagic enteritis virus (HEV) causes an acute viral disease in turkeys characterized by bloody diarrhea and death. Current live HEV virus vaccines are immunosuppressive and predispose turkeys to secondary bacterial infections. Data indicates that the capsid proteins (fiber, penton base, hexon) of HEV are capable of stimulating protective antibodies against an HEV challenge.

Using tobacco as a model, we sought to determine if a plant could be used to synthesize the HEV fiber protein and produce sufficient antigen to stimulate protective antibodies. To introduce the fiber gene into plants, the coding region of the HEV fiber gene was fused to either a constitutive plant promoter (35S) or a wound inducible promoter (hmg2) on plasmids adapted for *Agrobacterium*-mediated transformation. Approximately sixty transgenic plants of each construct were generated and determined to contain the HEV fiber gene based on amplification of specific HEV DNA sequences by the polymerase chain reaction. Plants were screened by Northern dot blot to identify lines expressing high levels of fiber mRNA. Expression of fiber protein was observed in selected lines of transgenic tobacco by Western blot analysis using turkey anti – HEV serum. The accumulation of fiber protein in leaves of tobacco transformants was quantified by Sandwich ELISA. Fiber protein from these plants has undergoing large – scale purification and concentration for a turkey immunization trials to determine if plant expressed fiber antigen is capable of inducing protective antibodies against HEV in turkeys.

DEDICATION

This thesis is dedicated to my great parents and husband,
Xuhai Zhang, Zhiqian Tian, and Yongqun He,
with my deepest love and gratitude

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LIST OF ABBREVIATIONS

Ag	antigen
Ab	antibody
BCIP	5-bromo-4-choloro-3-indolyl phosphate disodium
CaMV	cauliflower mosaic virus
CETAB	hexodecyltrimethyl ammonium
cm	centimeter
CPMV	cowpea mosaic virus
CsCl	cesium cloride
CT	cholera toxin
CT-B	cholera toxin subunit B
DIG	digoxigenin
DNA	deoxyribonucleic acid
EDTA	ethylenediamintetra acetic acid
ELISA	enzyme-linked immunosorbant assay
g	gram
HbsAg	hepatitis B surface antigen
HCl	hydrochloric acid
HE	hemorrhagic enteritis
HEV	hemorrhagic enteritis virus
HEV-A	hemorrhagic enteritis virus avirulent strain
HIV	human immunodeficiency virus
IgA	immunoglobulin A
IgG	immunoglobulin G
IPTG	isopropyl-beta-thiogalactopyranoside
i-rNV	insect cell made virus-like particle of Norwalk virus
Kb	kilobase pair
kDa	kilodalton
LT	heat labile enterotoxin
LT-B	heat labile enterotoxin subunit B
M	molar
mM	millimolar
mg	milligram
µg	microgram
$MgCl_2$	magnesium chloride
$MgSO_4$	magnisium sulfate
min	minute
ml	milliliter
µl	microliter
MOPS	3-N-morpholino proponesulfonic acid
m RNA	message ribonucleic acid
MS	murashige and skoog
NaCl	sodium chloride

NaOAc	sodium acetate
NBT	notri blue tetrazolium chloride
PBS	phosphate buffered saline
PCR	polymerase chain reaction
RNA	ribonucleic acid
SDS	sodium dodecylsulfate
SDS-PAGE	sodium dodecylsulfate polyacrylamide gel electrophoresis
SpaA	cell surface adhesion protein antigen A
TEV 5'UTR	tobacco etch virus leader sequence
T-DNA	transfer DNA
TGEV	transmissible gastroenteritis virus
TMV	tobacco mosaic virus
t-rNV	tobacco made virus-like particle of Norwalk virus
Tris	trizma base
UV	long wavelength ultraviolet light
V	volt

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