

**APPLICATION OF BIOTECHNOLOGY IN PLANT-  
BASED VACCINE PRODUCTION AGAINST  
HERPES SIMPLEX VIRUS**

By

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## ABSTRACT

**Aya Hussein Al-Turkey: Application of Biotechnology in Plant Based Vaccine Production Against Herpes Simplex Virus. Unpublished M.Sc. thesis, Department of Microbiology, Faculty of Agriculture, Ain Shams University, Egypt, 2015.**

Herpes simplex virus (HSV) is a DNA-viral genome and causing infection for both human and animal. HSV-2 is a sexually-transmitted virus. It is prevalence in European countries and also in Egyptian-closed societies i.e. in Upper Egypt.

Egyptian isolate of HSV-2 was isolated and its Glycoprotein D (gD) subunit was amplified, cloned and sequenced for characterization and detection. HSV-2gD subunit was amplified, using specific forward and reverse primers, with 1021 bp fragment size. The PCR product was cloned in One Shot Top 10 chemically competent *E.coli* cells using PCR 2.1/TOPO/ TA cloning vector. DNA insert was liberated from 1 µg recombinant plasmid by using the restriction endonuclease EcoR1. HSV-2 insert was successfully sub-cloned by ligation into purified binary vector PBI 121, using restriction endonucleases XbaI and T4 DNA ligase enzyme and transformed in DH5α competent *E.coli* cells.

*Agrobacterium tumefaciens* LBA 4404 strain competent cells were prepared. HSV-2gD subunit-containing binary vector PBI 121 were transformed into *Agrobacterium* for Agro-inoculation. Explants of Castell Rock and MPI tomato varieties were inoculated with the HSV-2gD-PB21 binary vector-treated *Agrobacterium* competent cells. Agro-inoculated tomato plants were tested for HSV-2gD insert occurrence by PCR. The expression of gD was confirmed through the RT\_PCR for the messenger RNA as well as ELISA for the expressed protein. The results were successful in getting transgenic tomatoes expressing the HSV-2gD as a step forward to develop an edible plant vaccine which is the aim of the study.

**Key words:**

Herpes simplex virus 2 –Polymerase chain reaction (PCR) – cloning – agro-inoculation–*Agrobacterium* Transformation - edible vaccine – tomato tissue culture – oral vaccines – gene expression – sexual transmitted disease.

## CONTENTS

	Page
<b>LIST OF TABLES .....</b>	<b>IV</b>
<b>LIST OF FIGURES .....</b>	<b>V</b>
<b>Abbreviation .....</b>	<b>VIII</b>
<b>INTRODUCTION .....</b>	<b>1</b>
<b>REVIEW OF LITRATURE .....</b>	<b>3</b>
<b>MATERIALS AND METHODS .....</b>	<b>45</b>
<b>Source of virus isolate.....</b>	<b>45</b>
<b>I. Genomic Material and Polymerase Chain Reaction.....</b>	<b>47</b>
<b>I.1.PCR amplification.....</b>	<b>47</b>
<b>I.2.Electrophoresis analyses.....</b>	<b>48</b>
<b>- Development of HSV-2 gD constructs.....</b>	<b>48</b>
<b>II.Molecular cloning.....</b>	<b>51</b>
<b>II.1.Ligation.....</b>	<b>51</b>
<b>II.2.Plasmidtransformation.....</b>	<b>51</b>
<b>II.3.Preparation of recombinant plasmids.....</b>	<b>52</b>
<b>II.4.Restriction digestion of plasmid DNA.....</b>	<b>53</b>
<b>II.5.DNA automatic sequencing.....</b>	<b>53</b>
<b>III.Molecular sub cloning.....</b>	<b>53</b>
<b>III.1. Creation of cloning sites.....</b>	<b>53</b>
<b>III.2. Gel extraction and plasmid purifcation.....</b>	<b>54</b>
<b>III.3. Ligation for sub-cloning.....</b>	<b>54</b>
<b>III.4.Comptent cell preparation.....</b>	<b>55</b>
<b>III.5.Transformation.....</b>	<b>56</b>

<b>I.V. <i>Agrobacterium</i> preparation and Tomato transformation.....</b>	<b>57</b>
<b>IV.1. Preparation of explants.....</b>	<b>57</b>
<b>IV.1.1. Sterilization of tomato seeds.....</b>	<b>57</b>
<b>IV.1.2. Seeds germination.....</b>	<b>57</b>
<b>IV.1.3. Preparation of explants for transformation.....</b>	<b>57</b>
<b>IV.2. Co-cultivation of explants with <i>Agrobacterium</i>.....</b>	<b>57</b>
<b>IV.2.1. Preparation of <i>Agrobacterium</i> competent Cell.....</b>	<b>57</b>
<b>IV.2.2. Transformation of <i>Agrobacterium</i> transformation competent cell by heat shock.....</b>	<b>58</b>
<b>-PCR Colony.....</b>	<b>58</b>
<b>IV.2.3. Preparation of <i>Agrobacterium</i> for co- cultivation.....</b>	<b>58</b>
<b>IV.2.4. Co-cultivation explants with <i>Agrobacterium</i>.....</b>	<b>59</b>
<b>IV.3. Regeneration and selection of transformed explants.....</b>	<b>59</b>
<b>IV.3.1. Regeneration.....</b>	<b>59</b>
<b>IV.3.2. Elongation.....</b>	<b>59</b>
<b>IV.3.3. Rooting.....</b>	<b>60</b>
<b>IV.3.4. Planting in soil and hardening.....</b>	<b>60</b>
<b>II.V. Analysis of the transgenic plants for successful Transformation...60</b>	
<b>II.V. 1. Screening for DNA using PCR.....</b>	<b>60</b>
<b>II.V.1.1. Isolation of total DNA from plant tissue.....</b>	<b>60</b>
<b>II.V.1.2. PCR.....</b>	<b>61</b>
<b>II.V.2.Expression analysis.....</b>	<b>61</b>
<b>II.V.2.1. Isolation of total RNA from plant tissue.....</b>	<b>61</b>
<b>II.V.2.2. One step RT-PCR.....</b>	<b>62</b>
<b>II.V.3Analysis for the protein expression.....</b>	<b>63</b>
<b>-ELISA.....</b>	<b>63</b>
<b>Solutions and buffers .....</b>	<b>64</b>

<b>RESULTS.....</b>	<b>67</b>
<b>DISCUSSION.....</b>	<b>90</b>
<b>SUMMARY.....</b>	<b>95</b>
<b>REFERENCES.....</b>	<b>98</b>
<b>ARABIC SUMMARY</b>	

## LIST OF TABLES

Table No.	Page
<b>1- Representative plant-based vaccines: under clinical development or in market (Kumar B <i>et al.</i>, 2013).....</b>	<b>23</b>
<b>2- Examples of recombinant protein (Bacterial Antigens) produced in <i>Solanum lycopersicum</i> in the last five years (2007–2012) (Maria Manuela Rigano <i>et al.</i>, 2013).....</b>	<b>32</b>
<b>3- Examples of recombinant protein (Viral Antigens) produced in <i>Solanum lycopersicum</i> in the last five years (2007–2012) (Maria Manuela Rigano <i>et al.</i>, 2013).....</b>	<b>34</b>
<b>4- Examples of recombinant protein (Human Antigens) produced in <i>Solanum lycopersicum</i> in the last five years (2007–2012) (Maria Manuela Rigano <i>et al.</i>, 2013).....</b>	<b>37</b>
<b>5- The amino acid expressed from HSV-2gD.....</b>	<b>75</b>

## LIST OF FIGURES

Fig. No.	Page
1-The multiple cloning sites (MCS) of the vector including the sites of the different restriction enzymes .....	49
2-A: The construct design in the plasmid. B: the features of PBI 121 vector and the sequence surrounding the PBI 121 Cloning site.....	50
3-Electrophoresis for PCR product of Egyptian isolates HSV-2 Gd.....	68
4- The minipreparation for pCR2.1-TOPO/HSV-2 cloning vector.....	69
5- The <i>EcoRI</i> digestion of the 2.1/TOPO/TA /HSV-2 glycoprotein D clones.....	70
6- HSV-2 glycoprotein D nucleotide sequence.....	72
7- Nucleotide sequence and derived amino acids.....	74
8- <i>XbaI</i> digestion for the TOPO /HSV-2 gD constructs and the linearized PBI 121 vector digested with <i>XbaI</i> enzyme digestion.....	77
9- HSV-2/gD gene cleane with QIAquick gel extraction kit.....	77
10- PBI 121 gene clean with PCI.....	78
11- The minipreparation for pCR2.1-TOPO/HSV-2 cloning vector...	78
12- PCR for sub clones to check the write orientation for the insert (glycoprotein D).....	79



<b>Fig. No.</b>	<b>Page</b>
<b>13- PCR colony for <i>Agrobacterium transformation</i> containing glycoprotein D.....</b>	<b>80</b>
<b>14-Germinated tomato seeds after 15 days old.....</b>	<b>81</b>
<b>15- Agro-inoculated tomato plant's cotyledons were arranged so that they tough each other.....</b>	<b>82</b>
<b>16- Agro-inoculated plants in the regeneration stage.....</b>	<b>83</b>
<b>17- Transformed tomato plants in elongation stage.....</b>	<b>84</b>
<b>18- Electrophoreses analysis for PCR amplification of the HSV-2/GD..</b>	<b>85</b>
<b>19- Electrophoreses analysis for RT-PCR amplification of the HSV-2/gD.....</b>	<b>86</b>
<b>20- ELISA plate coat with anti-gD antibody to capture the gD protein, showing positive results indicating that tomato transgenic plants infected with HSV-2gD produce the gD recombinant protein.....</b>	<b>87</b>
<b>21-The absorbance of each sample as a key for the ELISA plate A. HPC: Healthy plant control.....</b>	<b>88</b>
<b>22-Transformed Mp1 plants in the rooting stage.....</b>	<b>89</b>

### Abbreviation

A	Adenine
Agri	Agriculture
ARC	Agriculture research center
BSA	Bovine serum albumin
bp	base pair
CR	Castle Rock
C	Cytosine
°C	Centigrade
Dept	Department
DNA	Deoxyribonucleic acid
dNTP	Dideoxy nucleotide triphosphate
<i>E.Coli</i>	<i>Escherichia Coli</i>
EDTA	Ethylene diamine tetra acetic acid
<i>e.g</i>	For example (Exempli gratia)
ELISA	Enzyme Linked Immunesorbent Assay
<i>et al.</i>	And other ( <i>et alii</i> )
Fac.	Faculty
Fig.	Figure
g	Gram
G	Guanine
HCP	Healthy control plant
hr.	Hour
HBV	Hepatitis B virus
HIV	Human immunodeficiency virus
HSV-1	Herpes Simplex Virus-1
HSV-2	Herpes Simplex Virus-2
IAA	Indole acetic acid
IBA	Indol Butyric Acid
IgG	Immunoglobulin G
IPTG	IsopropyleB-D-thioglalacto Pyranoside
Kb	Kilobase

LB	Luria Borth
ml	Milliliter
mg	Milligram
min	Minute
μg	Microgram
μl	Microliter
M	Molar
MS	Murashige & Sookg
NCBI	National Center for Biotechnology Information
nt	Nucleotide
O.D	Optical density
PBS	phosphate buffere saline
PBST	phosphate buffere saline Tween-80
PCI	Phenol Chloroform Isoamyl Alcohol
PCR	Polymerase chain reaction
Res	Research
RT-PCR	Reverse transcriptase Polymerase chain reaction
rpm	Revolution per minutes
RNA	Ribonucleic acid
RNAse	RNAase inhibitor
s	second
SDS	Sodium dodecyle sulfate
STI	Sexually Transmitted Infection
TBE	Tris-boric acid-EDTA
T	Thiamine
U	Unit
Univ	University
UV	Ultraviolet
(v/v)	Volume/Volume
w/v	Weight/Volume
X-Gal	5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside

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## I. INTRODUCTION

Herpes simplex virus (HSV) infections of the skin are caused by one of two viruses (HSV-1 or HSV-2). Cutaneous *Herpes simplex* is characterized by painful, burning, or pruritic clusters of vesicles on the lips, oral mucous membranes, genital region, or other areas of the body. HSV infection of the eye results in keratoconjunctivitis, a serious condition that sometimes leads to corneal blindness. HSV may also cause encephalitis or other systemic infections, particularly in immune-compromised patients. Studies of vulnerable patient populations have indicated that daily use of antivirals such as acyclovir and valacyclovir can reduce reactivation rates (Koelle and Corey 2008).

After a primary infection, the virus travels to a nerve cell ganglion where it persists in a dormant phase. Various factors such as sun exposure, chapping or abrasion of the skin, fever, stress, fatigue, or menstruation can reactivate the virus, resulting in a recurrence at the site of the original infection. Recurrences are common, particularly in the case of genital infections. (Nahmias *et al.*, 1990; Chen *et al.*, 2000; Stanberry *et al.*, 2000; Wald *et al.*, 2000).

Genital herpes is associated with a two- to three-fold increased risk of HIV acquisition and an up to five-fold increased risk of HIV transmission per-sexual act, and may account for 40% to 60% of new HIV infections in populations where HSV-2 has a high prevalence. HIV, in turn, increases the risk of HSV-2 transmission. Outbreaks of HSV-2 are generally more severe, extensive, persistent, and invasive for those with more advanced HIV disease. In fact, persistent HSV-2 infection was one of the original opportunistic infections that resulted in the identification of AIDS (Corey *et al.*, 2004).

Genetic transformation of plant cells by *Agrobacterium tumefaciens* is the only known natural example of transkingdom DNA transfer. In nature, *Agrobacterium* introduces several oncogenic genes into the host plant, leading to formation of tumors, and in the laboratory this microorganism is

used widely for plant genetic engineering. *Agrobacterium* infection requires the presence of two genetic components located on the bacterial tumor-inducing (Ti) plasmid: the transferred DNA (T-DNA), which is introduced into the plant cell genome, and the virulence (*vir*) region composed of seven loci—*virA*, *virB*, *virC*, *virD*, *virE*, *virG*, and *virH* encoding most components of the protein apparatus for T-DNA transfer. The infection process begins by chemotactic attraction of *Agrobacterium* toward wounded sites on the host plant, attachment of the bacteria to the plant cell surface, and activation of the T-DNA transfer machinery. During the attachment, *Agrobacterium* first loosely binds to the plant surface, and then it produces cellulose fibrils that tighten the binding and allow attachment of additional bacteria (Matthysse *et al.*, 2000)

Tomato (*Lycopersicon esculentum* Mill.) is an important solanaceous vegetable crop grown throughout the world for its versatile uses. It is one of the most important protective foods as it possesses appreciable quantities of vitamins and minerals and sometime rightly referred to as poor man's orange (Devi *et al.*, 2008). It is grown throughout the country where irrigation water and arable land are available (Abdelmageed *et al.*, 2003). Currently, plant tissue culture of tomato used for many different purposes such as callus induction and plant regeneration. Plant regeneration is a key facilitator component in genetic transformations, using *Agrobacterium tumefaciens*, electroporation and particle bombardment. So, *In vitro* regeneration of cultivated tomato has been a subject of research because of the commercial value of the crop and its amenability for further re improvement via genetic manipulation (Evans 1989).

Transgenic plants are the plants in which foreign genes of desired characters have to be inserted. Transgenic plant have been found to have many advantages like, development of high yielding varieties of crop plants and disease resistant, and are plants with improved tolerance to biotic and abiotic stress. Moreover; transgenic plants can be used to develop edible vaccines against human viruses. To date, however, only a few vaccines are available for administration by these routes. A promising avenue to