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

By

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# ABSRTACT

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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 intopurified binary vector PBI 121, using restriction endonucleases XbaI and T4 DNA ligase enzyme and transformed in DH5α competent *E.coli* cells.

*Agrobacterium tumifaciens* 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.

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## Abbreviation

| | |
|---|---|
| A | Adenine |
| Agri | Agriculture |
| ARC | Agriculture research center |
| BSA | Bovine serum albumin |
| bp | base pair |
| CR | Castle Rock |
| C | Cytosine |
| $^{o}$C | Centigrade |
| Dept | Department |
| DNA | Deoxyribonucleic acid |
| dNTP | Dideoxy nucleotide triphosphate |
| *E.Coli* | *Escherichia Coli* |
| EDTA | Ethylene diamine tetra acetic acid |
| *e.g* | For example (Exampli gratia) |
| ELISA | Enzyme Linked Immunesorbent Assay |
| *et al.* | And other (*et alii*) |
| 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 acide |
| IBA | Indol Butyric Acid |
| IgG | Immunoglobin G |
| IPTG | IsopropyleB-D-thioglalacto Pyranoside |
| Kb | Kilobase |

| | |
|---|---|
| LB | Luria Borth |
| ml | Milliliter |
| mg | Milligram |
| min | Minute |
| µg | Microgram |
| µl | Microliter |
| M | Molar |
| MS | Murashinge & 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 tumifaciens* 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