

LE PIANTE COME BIOREATTORI

FFBTVMLE COWE BTOKEYUUKT

- POSSIBILI PRODOTTI

- Anticorpi
- Proteine di interesse farmaceutico
- Vaccini edibili

- Metaboliti secondari

- Polimeri biodegradabili

Produzione di Anticorpi

SISTEMI DI ESPRESSIONE

- Cellule di mammifero
- Latte di animali transgenici

SISTEMI DI ESPRESSIONE

- Cellule di mammifero

producono l'anticorpo "autentico"
soprattutto in termini di glicosilazione

SVANTAGGI: costi elevati
possibile contaminazione
con patogeni umani

- Latte di animali transgenici

alta resa; le proteine possono essere
recuperate periodicamente

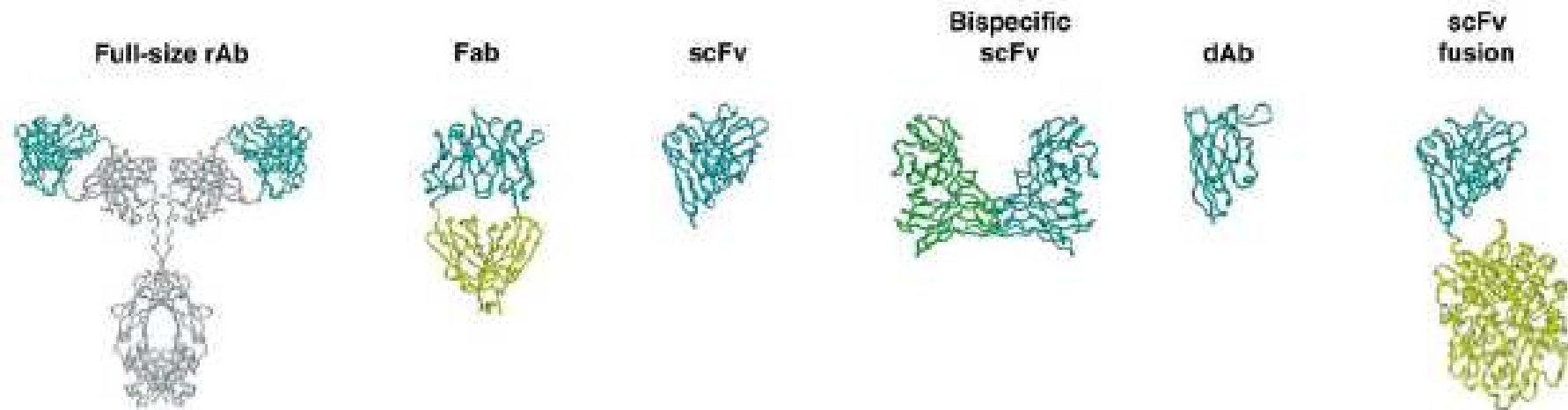
SVANTAGGI: necessità di produrre animali
transgenici –
possibile contaminazione con patogeni
umani

Produzione di Anticorpi in pianta "Plantibodies"

- **Vantaggi**

- Il costo per la produzione in pianta è molto minore che per la produzione in cellule animali
- La produzione nei semi rende possibile il mantenimento della proteina per lunghi periodi di tempo
- Non ci sono rischi di contaminazione con patogeni umani
- Il pathway di sintesi proteica è conservato tra piante e animali (corretto folding delle immunoglobuline)
- Le modificazioni post-traduzionali sono simili (glicosilazione)

anticorpi in pianta



tipi di anticorpi ricombinanti espressi in pianta

Preparazione del costrutto per l'espressione di anticorpi in pianta

- Isolamento dell'opportuno cDNA da una linea cellulare di ibridoma
- Inserimento nel vettore
 - promotore costitutivo:** **CaMV 35S** (dicotiledoni)
Ubiquitina (cereali)
 - promotore specifico per il seme**
(per evitare l'accumulo nei tessuti verdi qualora l'anticorpo interferisca con la crescita e lo sviluppo)

- Utilizzo di sequenze specifiche per il targeting cellulare

- ➡ anticorpi ricombinanti sono più stabili in alcuni compartimenti piuttosto che in altri
- ➡ le immunoglobuline full-length devono essere glicosilate: pathway di secrezione
- ➡ il targeting appropriato può facilitare l'isolamento e la purificazione

rese 10000 volte superiori se l'anticorpo è accumulato nell'ER

aggiunta della sequenza di ritenzione nell'ER (KDEL)

"Plantibodies"

scopo diagnostico e terapeutico

CaroRx

Application and specificity	Promoter	Signal sequences	Antibody name or type	Plant	Expression levels	Refs
Dental caries; streptococcal antigen I or II	CaMV 35S	Murine IgG signal peptides	Guy's 13 (SIgA) ^a	<i>Nicotiana tabacum</i>	500 µg/g FW ^b leaves	8,11
Diagnostic; anti-human IgG	CaMV 35S	Murine IgG signal peptides	C5-1 (IgG)	Alfalfa	1.0% TSP ^c	4
Cancer treatment; carcinoembryonic antigen	Maize ubiquitin	Murine IgG signal peptide; KDEL	ScFvT84.66 (ScFv)	Wheat	900.0 ng/g leaves; 1.5 µg/g seed	5
Cancer treatment; carcinoembryonic antigen	Maize ubiquitin	Murine IgG signal peptide; KDEL	ScFvT84.66 (ScFv)	Rice	29.0 µg/g leaves; 32.0 µg/g seed; 3.8 µg/g callus	5,6
Cancer treatment; carcinoembryonic antigen	Enhanced CaMV 35S	Murine IgG signal peptide; KDEL	ScFvT84.66 (ScFv)	Rice	27.0 µg/g leaves	5
Cancer treatment; carcinoembryonic antigen	Enhanced CaMV 35S	TMV Ω leader; murine IgG signal peptides; KDEL	T84.66 (IgG)	<i>Nicotiana tabacum</i> (transiently with <i>Agrobacterium</i> infiltration)	1.0 µg/g leaves	13
B-cell lymphoma treatment; idiotype vaccine	TMV subgenomic coat protein promoter	Rice α-amylase	38C13 (scFv)	<i>Nicotiana benthamiana</i>	30.0 µg/g leaves	9
Colon cancer; surface antigen	TMV subgenomic promoter U5 CP	Murine IgG signal peptide; KDEL	C017-1A (IgG)	<i>Nicotiana benthamiana</i>	Not reported	10
Herpes simplex virus 2	CaMV 35S	Tobacco extensin signal peptide	Anti-HSV-2 (IgG)	Soybean	Not reported	3

^aSIgA, secretory IgA.

^bFW, fresh weight.

^cTSP, total soluble protein.

CEA: glicoproteina tumorale
scopo diagnostico

applicazione topica previene la trasmissione del virus

**Espressione di anticorpi in piante per
aumentare la difesa nei confronti di
patogeni**

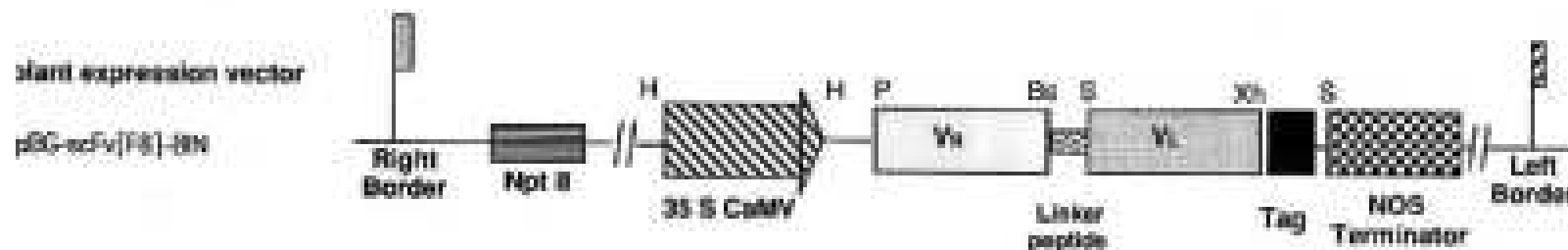
**Anticorpi diretti contro proteine di replicazione
o del capsido del virus possono interferire con
la replicazione e la diffusione del virus
aumentando la resistenza della pianta**

Transgenic plants expressing a functional single-chain Fv antibody are specifically protected from virus attack

Nature 1993

Paraskevi Tavladoraki⁺, Eugenio Benvenuto[†],
Stefania Trinca⁺, Domenico De Martinis⁺,
Antonino Cattaneo[‡] & Patrizia Galeffi⁺

espressione in piante di tabacco di
un anticorpo contro la proteina del
capside del virus dell' "arricciamento
maculato del carciofo"

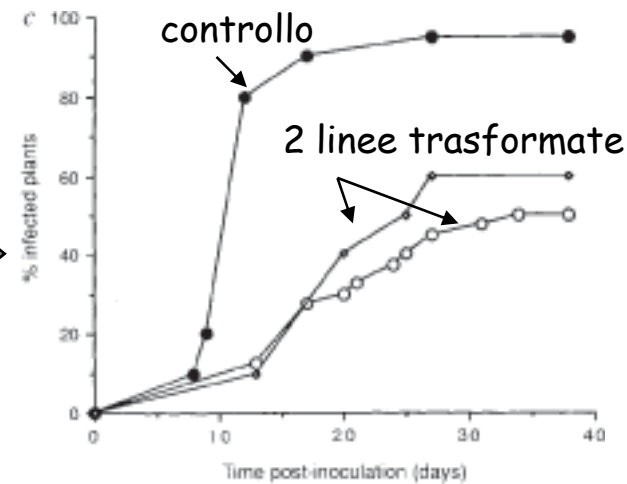
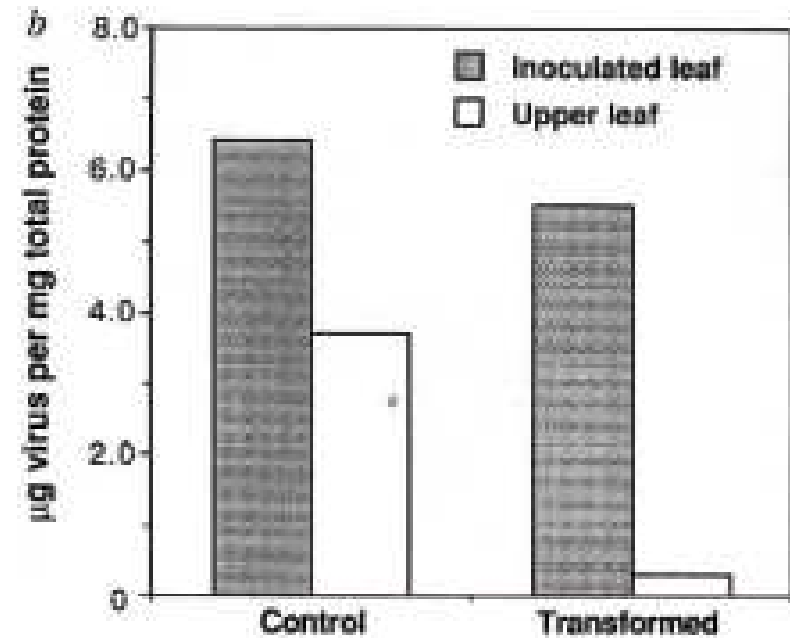


effetto dell'infezione virale in piante che esprimono l'anticorpo



trasformata controllo

% di piante infettate dopo
inoculazione con il virus



**Produzione di proteine di
interesse farmaceutico**

VANTAGGI

- ➔ L'**integrazione del DNA** nelle piante è stabile; nei microrganismi può non essere stabile
- ➔ Le **modificazioni post-traduzionali** sono più simili a quelle che hanno luogo nelle cellule animali
- ➔ Il **costo** per la produzione in pianta è molto minore che per la produzione in cellule animali
- ➔ La **produzione nei semi** rende possibile il mantenimento della proteina per lunghi periodi di tempo

SVANTAGGI

- ➔ la purificazione delle proteine da tessuto vegetale è molto più problematica che non da cellule animali

(presenza della parete, polifenoli - interferenza con le tecniche di purificazione)

E' necessario che i livelli di espressione siano molto elevati per avere rese soddisfacenti

Produzione di proteine di interesse farmaceutico

Pharmaceutical protein	Expression system	Expression level	Functional evaluation	Refs
Human growth hormone (hGH)	Rice suspension cells	57 mg l ⁻¹	The biological activity of shGH accumulated in the transgenic rice cell suspension culture was similar to that of the <i>E. coli</i> -derived recombinant hGH, as shown by proliferation of Nb2 node lymphoma cells	[88]
Mouse interleukin-12 (IL-12)	Tomato leaf and fruit	2.7–7.3 and 1–3.4 µg g ⁻¹ FW	Biologically active <i>in vitro</i> . The plant-produced mL-12 induced the secretion of IFN-γ by T cells	[89]
Human epidermal growth factor (hEGF)	Tobacco	0.09–0.3% TSP	Plant-produced hEGF significantly stimulated Vero E6 cell expansion and proliferation similar to commercial hEGF products	[90]
Human basic fibroblast growth factor (bFGF)	Soybean	2.3% TSP	Mitogenic assay demonstrated that bFGF stimulated Balb/c 3T3 cells to proliferate in a dose-dependent manner, indicating similar biological activity as native bFGF	[91]
Human acid β-glucosidase (GCase)	Tobacco seeds	Not reported	Plant-derived GCase was taken up by fibroblasts of a Gaucher type II patient and lacks potentially immunogenic glycans	[33]
Human glucocerebrosidase enzyme (GCD)	Carrot cells	Not reported	Recombinant GCD in transgenic plant cells is biologically active. Phase I clinical trial have shown that no clinical or laboratory evidence of any significant innate or humoral immune reactions. A Phase III clinical trial is currently ongoing	[8]
Human cytokine granulocyte macrophage colony stimulating factor (GM-CSF)	Sugarcane	Undetectable–0.02% TSP	Human bone marrow cells (TF-1), which require GM-CSF for cell division, proliferated when growth media was supplemented with transgenic sugarcane extracts and had identical activity levels	[92]
Human granulocyte-macrophage colony stimulating factor (hGM-CSF)	Rice	1.2–1.3% TSP	Rice seed-derived hGM-CSF induces proliferation of TF-1 cells similar to <i>E. coli</i> -derived hGM-CSF	[93]
Macrophage colony-stimulating factor (M-CSF) 1	Tobacco	0.02–1.92% TSP	Plant-derived M-CSFsR inhibits colony formation of J6-1 cells	[94]
Human granulocyte-colony stimulating factor (hG-CSF)	Rice suspension cells	0.7% TSP	Plant-derived hG-CSF supports proliferation of the AML-193 cells similar to commercial <i>E. coli</i> -derived hG-CSF	[95]
Bone morphogenetic protein 2 (BMP2)	Tobacco	0.02% TSP	Application of hBMP2 to mouse C2C12 cell line significantly increased cell ALP activity but is lower than commercial rhBMP2	[96]
Human α-1-antitrypsin (AAT)	Tomato	0.44–1.55% TSP	Biologically active, showing high specific activity and efficient inhibition of elastase activity	[97]
Human IA-2 (IA-2ic), a diabetes-associated autoantigen	Tobacco (Transient)	0.5% TSP	Plant-derived IA-2ic protein is specifically recognized by human IA-2ic autoantibodies	[35]
Human fibroblast growth factor 8 isoform b (FGF8b)	Tobacco (transient)	90–150 µg g ⁻¹ FW	Plant-expressed FGF8b effectively increased the rate of cell proliferation of NIH3T3 as bacterially expressed mouse FGF8b	[36]

A Plant-Derived Recombinant Human Glucocerebrosidase Enzyme—A Preclinical and Phase I Investigation

David Aviezer¹, Einat Brill-Almon¹, Yoseph Shaaltiel¹, Sharon Hashmueli¹, Daniel Bartfeld¹, Sarah Mizrachi², Yael Liberman², Arnold Freeman², Ari Zimran³, Eithan Galun^{2*}

¹ Protalix Biotherapeutics, Science Park, Carmiel, Israel, ² Goldyne Savad Institute of Gene Therapy, Hadassah-Hebrew University Hospital, Jerusalem, Israel, ³ Gaucher Clinic, Shaare Zedek Medical Center, Jerusalem, Israel

Malattia di Gaucher

Alterazione del metabolismo degli sfingolipidi

Accumulo di glucosilceramide nei lisosomi dei macrofagi

proteine di interesse
farmaceutico

Per aumentare la resa della purificazione si possono esprimere **proteine di fusione con tag** che permettano una facile purificazione mediante **cromatografia di affinità**

sito proteolitico



proteine di interesse
farmaceutico



trasformazione *Brassica napus*



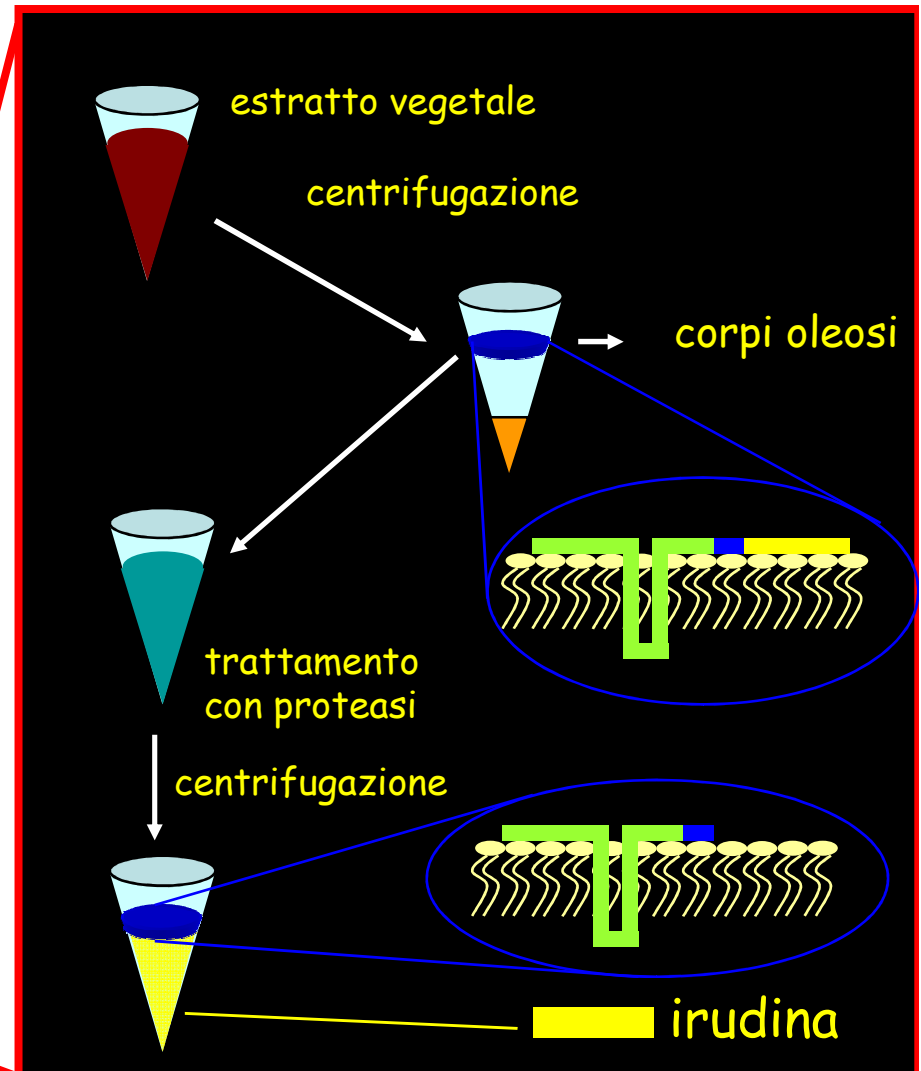
espressione nei corpi oleosi



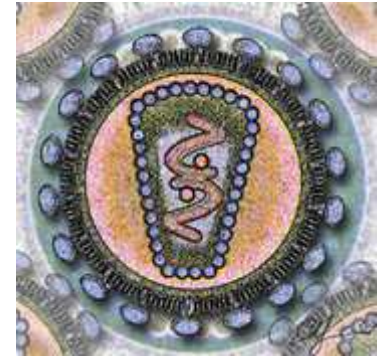
purificazione dei corpi oleosi



digestione proteolitica



VACCINI EDIBILI



- I vaccini attuali sono costosi
- Sono richieste infrastrutture per il trasporto e la somministrazione (problemi logistici ed economici)



Problema per i paesi poveri

Vantaggi della produzione in piante transgeniche:

Coltivazione in loco; basso costo

Non è necessario personale per la somministrazione del preparato

Disponibili indefinitamente (ogni anno si riseminano)

VACCINI EDIBILI

Technical and social benefits envisaged in plant-derived edible vaccines

No.	Benefit	Characteristics
1	Oral delivery	The plant cell wall, consisting essentially of cellulose and sugars, provides protection in the stomach and gradual release of the antigen in the gut
2	Use as raw food or dry powder	The vaccinogenic plant tissue may be used as raw food, dried or, alternatively, proteins may be partially or fully purified and administered in capsules as dry powder
3	No need for "cold chain"	The vaccinogenic plant parts or plant extracts can be stored and shipped at room temperature
4	Mucosal and serum immune response	Plant-derived vaccines are primarily designed to trigger the mucosal immune system (IgA), thus preventing pathogen entry at mucosal surfaces; they also elicit serum and, possibly, cytotoxic responses
5	Cost efficiency	Production cost will be reduced 100–1000 times as compared with that of traditional vaccines
6	Optimised expression system	Plants may be engineered to accumulate the antigen in convenient intracellular compartments (endoplasmic reticulum, chloroplast)
7	Ease of genetic manipulation	Procedures essentially rely on established molecular and genetic manipulation protocols; these are already available in developing countries
8	Ease of production and scale-up	GM-plants can be stored as seeds. Unlimited vaccine quantity can be produced from these in limited time; production and management is suitable for developing countries
9	Safer than conventional vaccines	Lack of contamination with mammalian pathogens
10	Ideal to face bio-weapons	Safety and cost efficiency propose plants plant-derived vaccines as an ideal tool to face bio-terrorism
11	Ideal for veterinary use	Cost affordable Ready for use as food additive

Trasformazione stabile

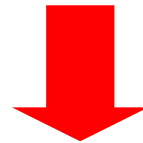
Espressione transiente



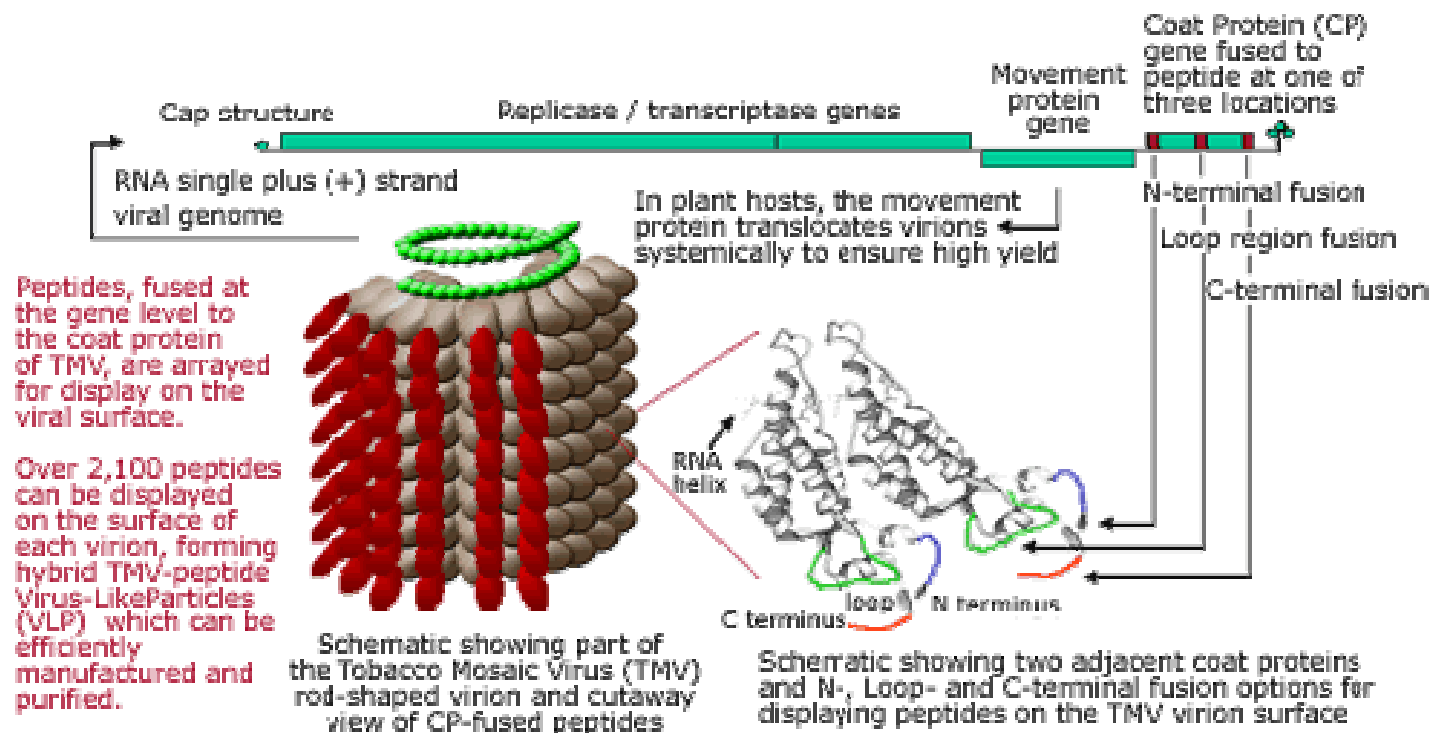
VETTORI VIRALI

Sistema di espressione del polipeptide

Sistema di presentazione dell'epitopo



LSBC'S CP Fusion/Virus-Like Particle (VLP) Viral Vectors



Cholera toxin B protein in transgenic tomato fruit induces systemic immune response in mice

Xiao-Ling Jiang · Zhu-Mei He ·
Zhi-Qiang Peng · Yu Qi · Qing Chen ·
Shou-Yi Yu

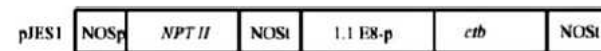
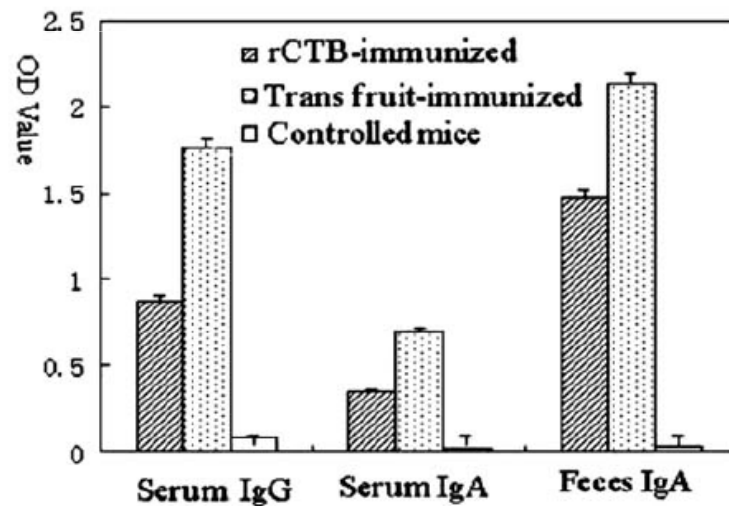


Fig. 1 Schematic representation of plasmid pJES1 used for tomato transformation NOS-p: nopaline synthase promoter; NOS-t: nopaline synthase terminator; *NPT II*: neomycin phosphotransferase II gene



Topi nutriti con i pomodori transgenici risultano immunizzati e producono sia IgG che IgA

RESEARCH ARTICLES

Production of hepatitis B surface antigen in transgenic plants for oral immunization

Liz J. Richter¹, Yasmin Thanavala², Charles J. Arntzen¹, and Hugh S. Mason^{1*}

¹Boyce Thompson Institute for Plant Research, Inc., Tower Rd., Ithaca, NY 14853-1801. ²Department of Immunology, Roswell Park Cancer Institute, 14263. *Corresponding author (HSM7@cornell.edu).

A



B

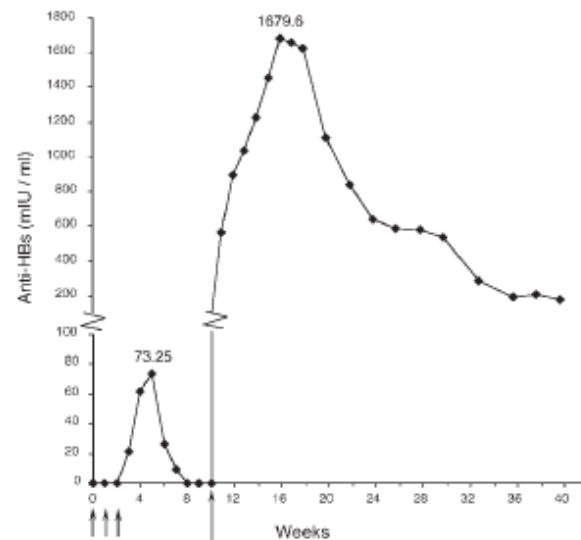


Figure 1. Oral immunization of mice with transgenic potatoes expressing HBsAg. (A) Structure of T-DNA region of pPAT-HB used to create the transgenic line PAT-HB-7. LB, RB: left and right border elements of the T-DNA that is mobilized into plant cells by *Agrobacterium tumefaciens*; ATG, start codon; TGA, stop codon. (B) Serum immune responses of mice that were fed raw tubers of potato line PAT-HB-7 expressing 1.1 μ g HBsAg/g tuber. Animals were fed 5 g potatoes plus 10 μ g CT per mouse per feeding. Small arrows indicate the feeding schedule: day 0, 7, and 14. Large arrow indicates a single intraperitoneal boost at week 10 with alum-adsorbed recombinant HBsAg (0.5 μ g/mouse). Data are means of values for 10 animals. Numbers above peaks indicate the anti-HBsAg titer.

Proteine espresse in piante transgeniche con applicazioni come vaccini animali e umani

Source of the protein and target species for the vaccine	Protein or peptide expressed	Plant expression system	Maximum recorded expression level in <i>planta</i>	Integrity, immunogenicity and protective capacity of the vaccine	Refs
Enterotoxigenic <i>E. coli</i> (humans)	Heat-labile toxin B-subunit	Tobacco	<0.01% TSP ^a	Intact protein forms multimers and is immunogenic when administered orally	14
Enterotoxigenic <i>E. coli</i> (humans)	Heat-labile toxin B-subunit	Potato	0.19% TSP	Receptor-binding activity and immunogenic and protective when administered orally	14,22,25
Enterotoxigenic <i>E. coli</i> (humans)	Heat-labile toxin B-subunit	Maize	Not given	Immunogenic and protective when administered orally	30
<i>Vibrio cholerae</i> (humans)	Cholera toxin B-subunit	Potato	0.30% TSP	Intact protein forms multimers, has receptor-binding activity and is immunogenic and protective when administered orally	18,19
Hepatitis B virus (humans)	Envelope surface protein	Tobacco	<0.01% TSP	Virus-like particles form and extracted protein is immunogenic when administered by injection	15,23
Hepatitis B virus (humans)	Envelope surface protein	Potato	<0.01% FW ^b	Immunogenic when administered orally	31
Hepatitis B virus (humans)	Envelope surface protein	Lupin (<i>Lupinus</i> spp.)	<0.01% FW	Immunogenic when administered orally	27
Hepatitis B virus (humans)	Envelope surface protein	Lettuce	<0.01% FW	Immunogenic when administered orally	27
Norwalk virus (humans)	Capsid protein	Tobacco	0.23% TSP	Intact protein and virus-like particles form, immunogenic when administered orally	16
Norwalk virus (humans)	Capsid protein	Potato	0.37% TSP	Virus-like particles form and immunogenic when administered orally	16,26
Rabies virus (humans)	Glycoprotein	Tomato	1.00% TSP	Intact protein	17
Human cytomegalovirus (humans)	Glycoprotein B	Tobacco	<0.02% TSP	Immunologically related protein	32
Rabbit hemorrhagic disease virus (rabbits)	VP60	Potato	0.30% TSP	Immunogenic and protective when administered by injection	28
Foot-and-mouth disease virus (agricultural domestic animals)	VP1	<i>Arabidopsis</i>	Not given	Immunogenic and protective when administered by injection	20
Foot-and-mouth disease virus (agricultural domestic animals)	VP1	Alfalfa	Not given	Immunogenic and protective when administered by injection or orally	24
Transmissible gastroenteritis coronavirus (pigs)	Glycoprotein S	<i>Arabidopsis</i>	0.06% TSP	Immunogenic when administered by injection	21
Transmissible gastroenteritis coronavirus (pigs)	Glycoprotein S	Tobacco	0.20% TSP	Intact protein and immunogenic when administered by injection	29
Transmissible gastroenteritis coronavirus (pigs)	Glycoprotein S	Maize	<0.01% FW	Protective when administered orally	30

^aTSP, total soluble protein.
^bFW, fresh weight.

SVANTAGGI DEI VACCINI EDIBILI

- ▶ le piante sono organismi viventi che cambiano: la continuità nella produzione del vaccino può non essere garantita
- ▶ i vaccini edibili possono essere confusi con frutti normali e consumati senza controllo
- ▶ il dosaggio è variabile e difficile da controllare: frutti di dimensioni diverse conterranno quantità diverse di antigene
- ▶ il pattern di glicosilazione è simile, ma non identico, a quello delle cellule animali; ciò può influenzare la funzionalità del vaccino

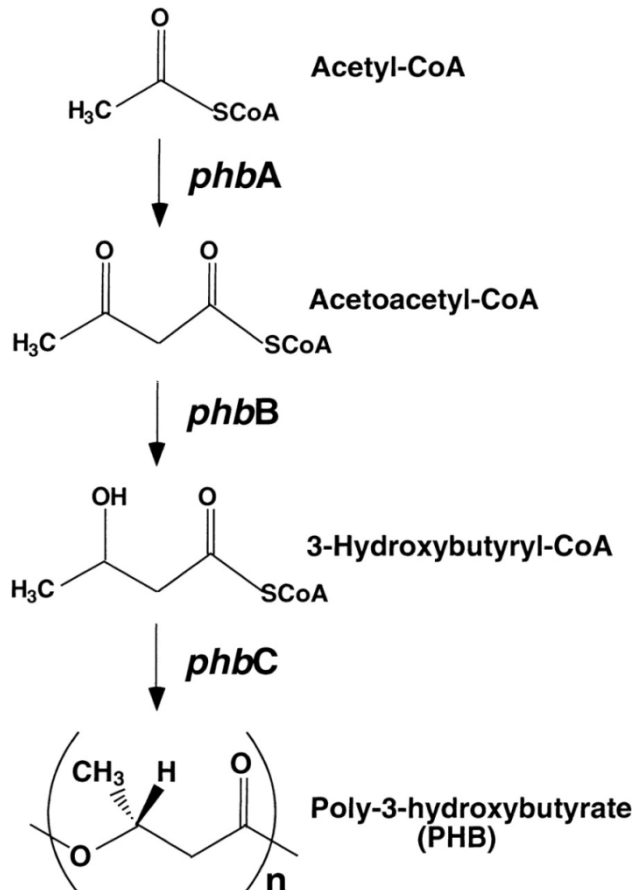
ΕΚΔΟΣΗ: 01.07.2015

La plastica biodegradabile POLI-IDROSSI-BUTIRRATO (PHB) è prodotta per fermentazione dal batterio *Alcaligenes eutrophus*. Il PHB è biodegradabile, ma impermeabile all'acqua: è però molto più costoso della plastica convenzionale.



Production of Polyhydroxybutyrate by Polycistronic Expression of Bacterial Genes in Tobacco Plastid

Yuko Arai^{1,3,5,6}, Toshiharu Shikanai^{4,7}, Yoshiharu Doi³, Shigeo Yoshida², Isamu Yamaguchi^{1,8} and Hideo Nakashita^{1,2,5,9}



Operone batterico *phb*

phbA: β-chetotilasi

phbB: AcetoacetylCoA-reduttasi

phbC: PHB polimerasi

