Production de molécules d'interêt par les plantes

'Plant Molecular Farming'

Protéines

Métabolites

UE MV2.5: Biotechnologie Microbienne et Végétale - Pr. Christophe Robaglia

Cécile Vriet -19 Février 2013

1. Production de protéines recombinantes

Pourquoi des protéins en thérapeutique?

Certaines cibles (récepteurs) ne répondent qu'à des effecteurs de structure plus complexe que des molécules «simples » issus de la chimie:

- Coagulation et facteur VIII
- Croissance et hGH
- Glycémie et insuline

Nécessité d'avoir recours, en thérapeutique, à des macromolécules, le plus souvent des protéines

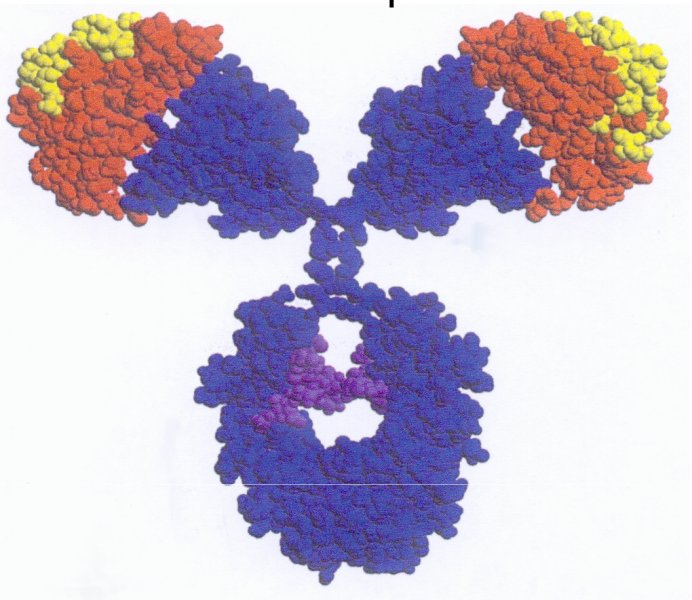
1. Production de protéines recombinantes

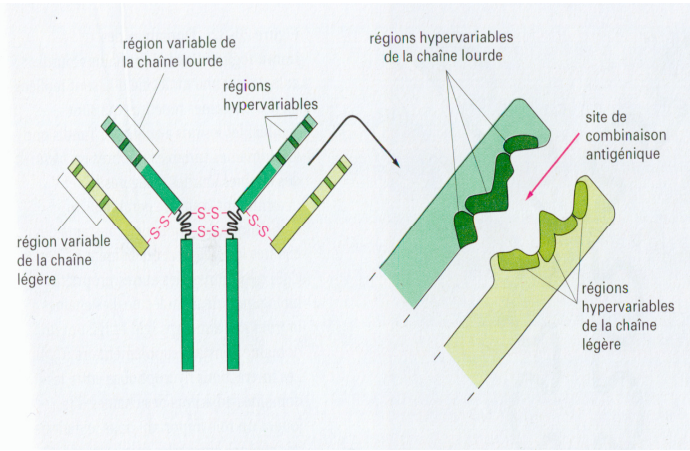
- Protéine =
  - structure complexe
  - Non accessible par la chimie de synthèse
  - Extraction
    - Tissus/fluides animaux
    - Tissus/fluides humains

- Limitations pour l'approvisionnement :
  - Accessibilité /disponibilité
  - Barrière d'espèce
  - Sécurité (micro)biologique

- Nécessité de nouvelles sources ?
  - Recours au génie génétique et OGM

Anticorps



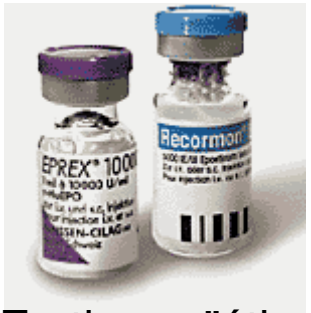


Production de protéines en milieu confiné:

- organismes:
  - bactéries,
  - levures,
  - cellules de mammifère (eg. CHO, ‘Chinese hamster ovary’ cells)
- Milieu: systèmes clos
  - Fermenteur
  - Cytoculteur
- Production : protéines médicaments (voir liste)

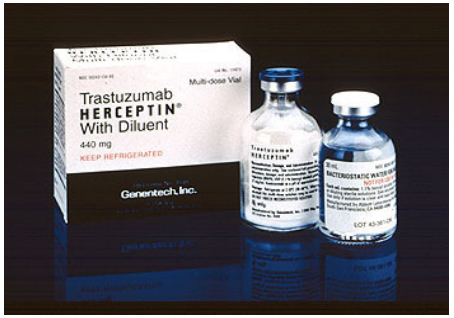
Exemples de protéines recombinantes commercialisées depuis 1987:

- Hormones
  - Hormone de croissance (somatropine, hGH)
  - FSH, LH
  - Insuline
  - Glucagon
- Cytokines
  - Interférons (alpha, beta, gamma)
  - Interféron consensus (alpha)
  - CSF (G- ; GM-)
  - IL2
  - EPO
- Coagulation
  - tPA
  - F. VII
  - F. VIII
  - F. IX
  - Hirudine
- Anticorps monoclonaux
  - Diagnostic (anti-CEA)
  - Anti-TNF
  - Anti-Lymphocyte
- Divers
  - Vaccin hépatite B
  - DNAse



Erythropoïétine (EPO)

Hormone de croissance



Anticorps monoclonaux

1. Production de protéines recombinantes

Avantages

- Procédés de production maîtrisés
  - Reproductibilité / homogénéité
  - Système clos

Inconvénients

- Modifications post-traductionnelles variables selon l'organisme producteur
  - glycosylation (ajout de sucres a des protéines qui dépendent de l'organisme)

- Volume/quantité de production limité

- Coût élevé
  - Investissement important
  - Développement long
  - Rendement +/- faible

Organismes producteurs de médicaments en système ouvert: les animaux transgéniques

- Vache, chèvre, brebis, souris, cochon, lapin…
- Elevage en étables contrôlées
- Protéine purifiée à partir de tissu/fluide animal (ex. lait)
- Example: Antithrombine III

Animaux transgéniques

Avantages

- Modifications post-traductionnelles des protéines similaires à l'humain
- Capacité de production relativement importante
  - Récolte facile (lait)
  - Rendement intéressant

Inconvénients

- Sécurité virale
  - Barrière d'espèce?
- Risque de contamination
  - Biologique
  - Physico-chimique
- Développement difficile
  - Maturation animale longue
  - Clonage?

Organismes producteurs de médicaments en système ouvert: les plantes transgéniques

- Maïs, tabac, tomate, pomme de terre, luzerne, colza…
- Culture en serres ou en plein champ
- Pas de médicament encore commercialisé, mais de nombreux en cours de développement
- Examples:

  - Albumine humaine, glucocérébrase humaine, Lactoferrine, Lipase gastrique

  - Vaccins et Anticorps recombinants (Medicago Inc., Planet Biotechnology Inc.)

1. Production de protéines recombinantes

Plant molecular farming - Examples de companies

Table 2
Examples of the three classes of recombinant proteins being produced using plants.

Classification	Recombinant proteins	Plant expression platform	References
Therapeutic proteins	**Antibody**: anti-West Nile virus mAb Hu-E16[a], Guy's 13 SIgA (CaroRx)[b]	*Nicotiana benthamiana*	Lai et al. (2010), Ma et al. (1998)
	Vaccine: H5N1, H1N1[c]	*N. benthamiana*	Shoji et al. (2008, 2011)
	Therapeutic enzyme: glucocerebrosidase[d]	Carrot suspension cells	Aviezer et al. (2009a, 2009b)
	Blood protein: human serum albumin[e]	Potato	Sijmons et al. (1990)
	Cytokine: interlukin-12	Tobacco hairy roots	Liu et al. (2009)
	Growth factor: human epidermal growth factor	Tobacco tissues	Parsons et al. (2010)
	Growth hormone: human growth hormone[f]	Tobacco chloroplast	Staub et al. (2000)
	Oral therapeutic: human intrinsic factor[g]	*Arabidopsis thaliana*	Fedosov et al. (2003)
Industrial enzymes	Cellulase	Corn	Hood et al. (2007, 2011)
	β-glucuronidase[h]	Corn	Kusnadi et al. (1998)
	Trypsin[h]	Corn	Woodard et al. (2003)
	Avidin[h]	Corn	Hood et al. (1997), Murray et al. (2002)
	α-Amylase[i]	Corn, tobacco	Pen et al. (1992)
	Laccase	Corn	Hood et al. (2003)
Biopolymers	Spider silk proteins	Tobacco, potato, *Arabidopsis*	Menassa et al. (2004), Scheller et al. (2001), Yang et al. (2005)
	Elastin-like polypeptides	Tobacco	Conley et al. (2009)
	Collagens[j]	Tobacco, corn	Ruggiero et al. (2000), Xu et al. (2011b)
	Plant gum	Tobacco suspension cells	Xu et al. (2005)

[a] Recent on commercial route.
[b] Phase II clinical trial completed by Planet Biotechnology; approved for use in the EU, but not marketed.
[c] Phase II and phase I clinical trials for H5N1 and H1N1, respectively, completed by Medicago with positive results.
[d] Phase III clinical trial completed by Protalix; the first plant-made therapeutic protein entering commercial sector; FDA approval pending.
[e] First complex human protein expressed in plant.
[f] Early demonstration of high-yield protein expression in plant chloroplast.
[g] Phase II clinical trial completed by Cobento Biotech AS; Marketed in the EU.
[h] Sigma Aldrich products come as research enzyme or drug.
[i] Recently (Feb, 2011) commercialized by Syngenta.
[j] Under preclinical development by Medicago and Meristem Therapeutics.

Xu et al, 2012 (Biotechnology Advances 30)

Plantes transgéniques

Avantages

- Capacité de production importante
- Coût / rendement attractif
- Sécurité virale accrue
- Production simple dans tous les pays
- grande uniformité de production (clonage naturel)

Inconvénients

- Glycosylation et modifications post-traductionnelles
- Paramètres de culture non maîtrisables
- Risque de contamination
  - Biologique (bactéries du sol)
  - Physico-chimique (sous produits de la plante)
- Risque de dissémination (pollen)?

Comparatif des systèmes d'expression

Table 1. Comparison of features of recombinant protein production in plants, yeast and classical systems

	Transgenic plants	Plant viruses	Yeast	Bacteria	Mammalian cell cultures	Transgenic animals
Cost/storage	Cheap/RT	Cheap/−20°C	Cheap/−20°C	Cheap/−20°C	expensive/N_2	Expensive
Distribution	Easy	Easy	Feasible	Feasible	Difficult	Difficult
Gene size	Not limited	Limited	Unknown	Unknown	Limited	Limited
Glycosylation	'Correct' ?	'Correct' ?	Incorrect	Absent	'Correct'	'Correct'
Multimeric protein assembly (SIgA)	Yes	No	No	No	No	Yes
Production cost	Low	Low	Medium	Medium	High	High
Production scale	Worldwide	Worldwide	Limited	Limited	Limited	Limited
Production vehicle	Yes	Yes	Yes	Yes	Yes	Yes
Propagation	Easy	Feasible	Easy	Easy	Hard	Feasible
Protein folding accuracy	High ?	High ?	Medium	Low	High	high
Protein homogeneity	High ?	Medium	Medium	Low	Medium	Low
Protein yield	High	Very high	High	Medium	Medium-high	high
Public perception of 'risk'	High	High	Medium	Low	Medium	High
Safety	High	High	Unknown	Low	Medium	High
Scale up costs	Low (unlimited biomass)	Low	High**	High**	High**	High
Therapeutic risk*	Unknown	Unknown	Unknown	Yes	Yes	Yes
Time required	Medium	Low	Medium	Low	High	High

* – residual viral sequences, oncogenes, endotoxins; ** – large, expensive fermenters etc; ? – unclear.

Fischer et Emans, 2000 (Transgenic Research)

1. Production de protéines recombinantes

• **Critères de choix - à coût équivalent - des systèmes de production de protéines recombinantes** :

- du type de protéine a produire (organisme d'origine, facteur soluble ou pas…)

- du rendement à atteindre (petite versus grande échelle)

- du niveau de pureté nécessaire, des possibilité de stockage,…

• **Succès commercial de la production de protéines recombinante** dépend:

- des considérations économiques: coût de la production (dépend du rendement) et coût de l'extraction/purification ('downstream processing')

- de la technologie: efficacité, facilité de mise en place, 'scalabilité', etc…

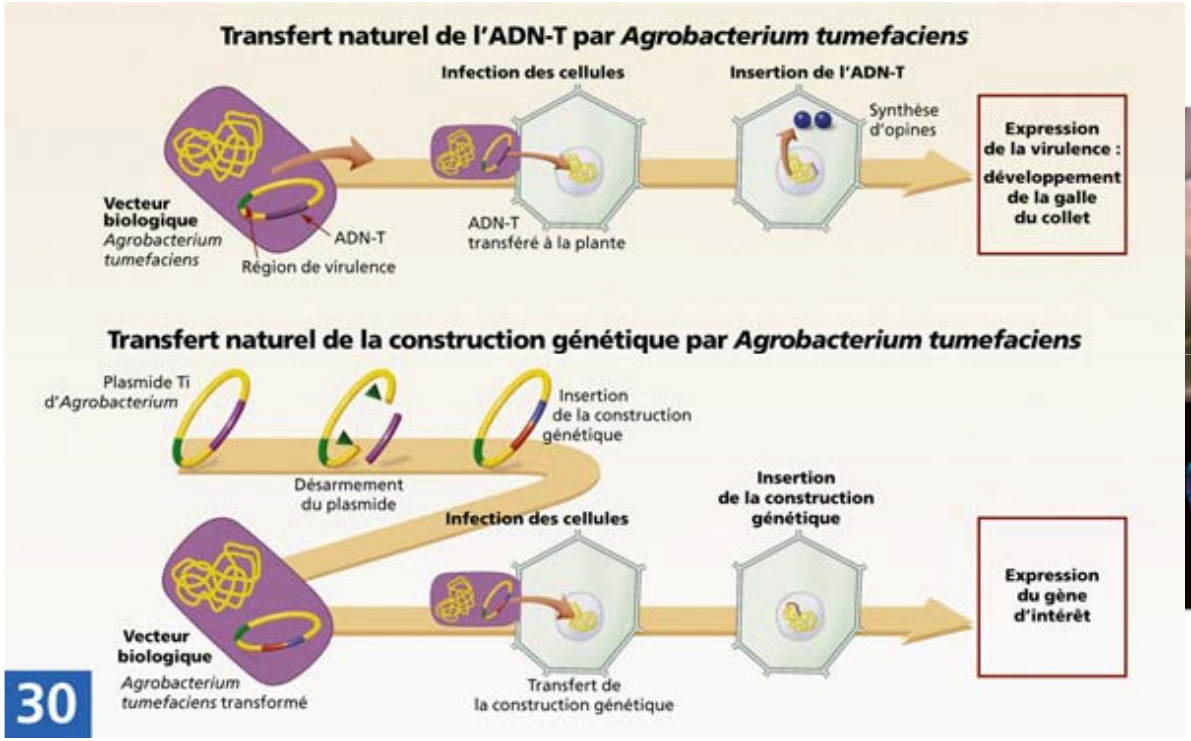
Mais aussi….

- des aspects de propriété industrielle, de la facilité de dé-régulation (sécurité biologique) et de mise sur le marché

- du risque perçu/de l' acceptation par l'opinion publique!

2. Production de protéines recombinantes chez les plantes

Expression stable ou transitoire utilisant *Agrobacterium Tumefaciens*



Expression transitoire utilisant des vecteurs viraux réplicatifs

TRBO: A High-Efficiency Tobacco Mosaic Virus RNA-Based Overexpression Vector[1][C][OA]

John A. Lindbo*

Department of Plant Pathology, The Ohio State University/Ohio Agricultural Research and Development

Le virus est introduit par Agroinfection

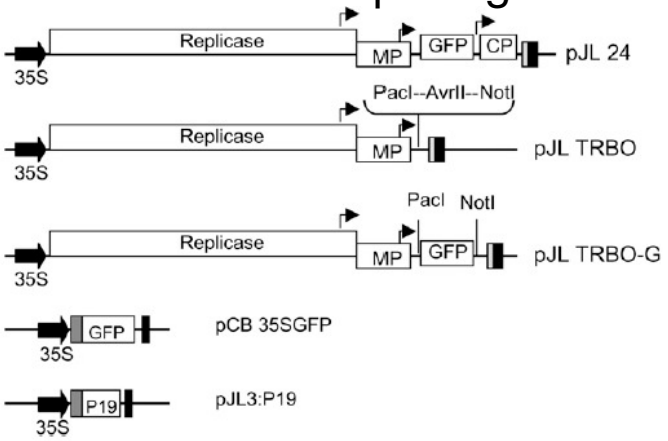


Figure 1. Maps of plasmids used in this project. The T-DNA regions of binary plasmids used in this project are represented. Block arrow, CaMV duplicated 35S promoter. Black box, CaMV polyA signal sequence/terminator. Dark gray box, Tobacco etch virus 5′-nontranslated leader sequence. Light gray box, Ribozyme. Bent arrows, Subgenomic promoters. ORFs are represented by white boxes. Identities of ORFs are labeled in white boxes. Replicase, TMV 126K/183K ORF; MP, movement protein; P19, 19-kD RNA-silencing suppressor gene from *Tomato bushy stunt virus*.

Il reste confiné aux feuilles inoculées

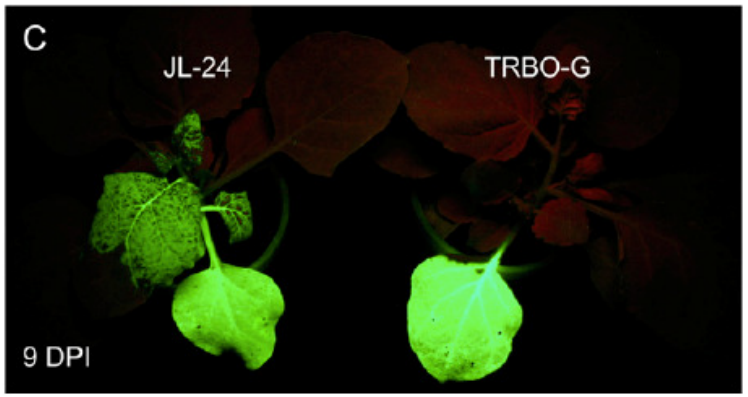


Figure 4. TRBO-G replicon does not move systemically in plants. One leaf of an *N. benthamiana* plant was infiltrated with *A. tumefaciens* carrying pJL24 or pJL-TRBO-G plasmids. Plants were photographed under UV light to visualize the GFP expressed by either expression vector. [See online article for color version of this figure.]

Optimisation : Augmenter le niveau d'expression

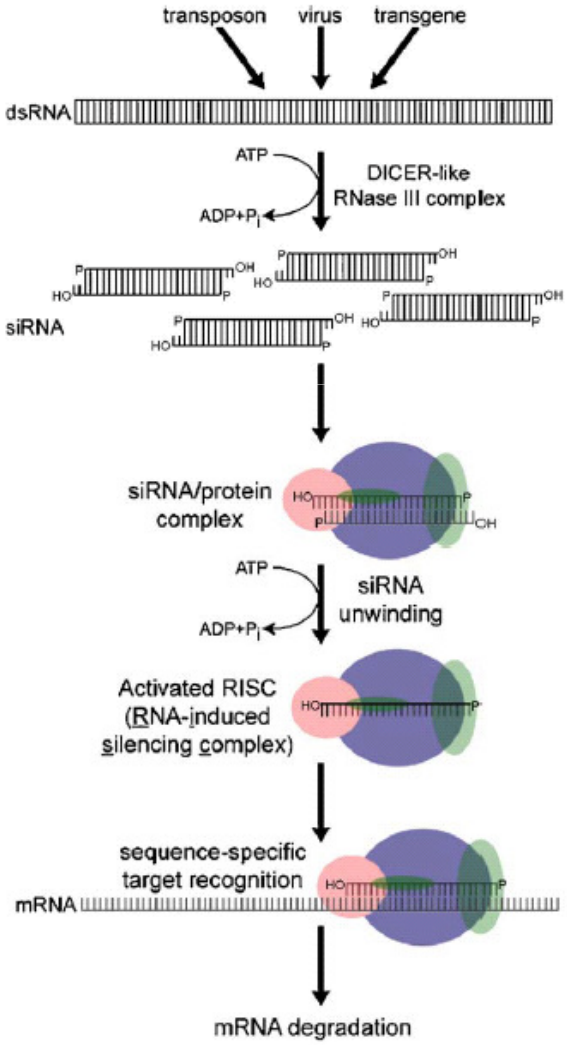
• **Promoteur:**
- 35S pour les eudicots; Ubiquitin + introns pour les monocots
- regulés/induits vs constitutifs

• **Codon usage**

• **Combattre le RNA silencing**:

- mécanisme de dégradation spécifique de séquence de l'ARN
, activé par l'ARN double brin et ARN « aberrants »

- protection contre les acides nucléiques invasifs (virus, transposons, transgènes)



2a. Techniques d'expression chez les plantes

Combattre le RNA silencing

Des UTR viraux et l'inhibition du RNA silencing permettent une expression <u>transitoire</u> très élevée (> 1g/kgFW)

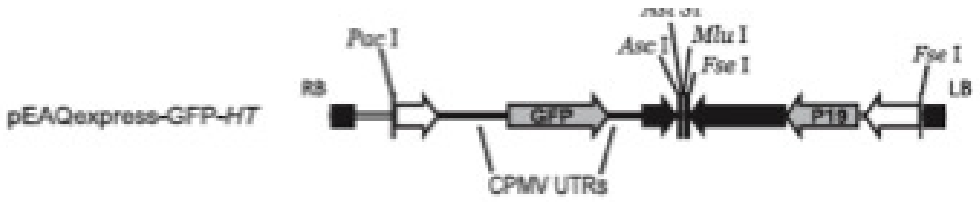
Plant Biotechnology Journal

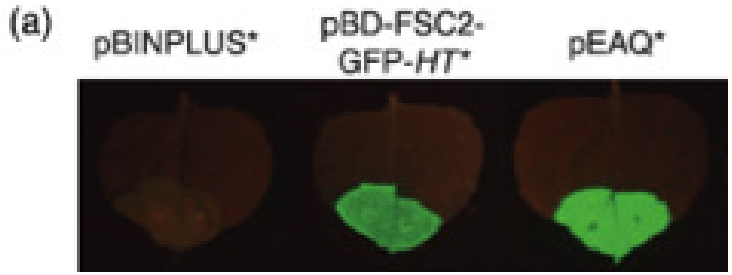
Plant Biotechnology Journal (2009) **7**, pp. 682–693 doi: 10.1111/j.1467-7652.2009.00434.x

pEAQ: versatile expression vectors for easy and quick transient expression of heterologous proteins in plants

Frank Sainsbury†, Eva C. Thuenemann† and George P. Lomonossoff*

Department of Biological Chemistry, John Innes Centre, Norwich, NR4 7UH UK





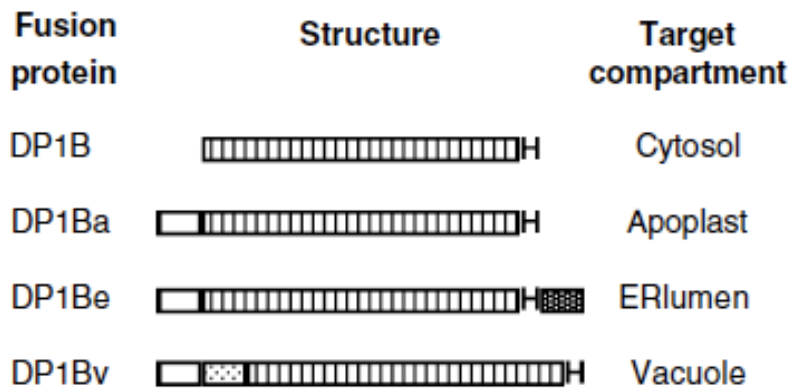
P19: protéine virale seéquestrant les siARNs

Optimisation : Adressage des protéines

Transgenic Research (2005) 14:313–324
DOI 10.1007/s11248-005-0272-5

High yield recombinant silk-like protein production in transgenic plants through protein targeting

(a)



(c)

Construct	Number of plants	Yield range (% TSP)	Average yield (% TSP)	Productivity (fold)
pGY411	4	1.1-1.4	1.2	1
pGYV511	5	None	None	None
pGYV512	25	1.8-18	9.4	7.8
pGYV513	6	4.8-8.2	6.5	5.4

cytosol
Apoplaste
ER lumen
vacuole

Optimisation : transformation chloroplastique

Interêt de la transformation génétique chloroplastique:

-10.000 copies d’ADN chloroplastique par cellule , vs 2-6 pour le genome nucleaire

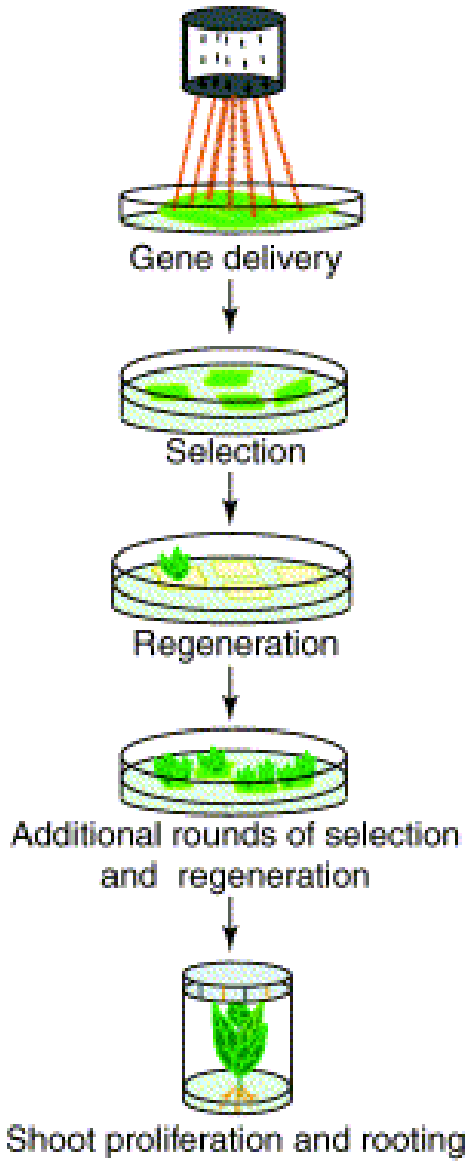
- Niveau d’expression élevé: 40% des protéines de la feuille

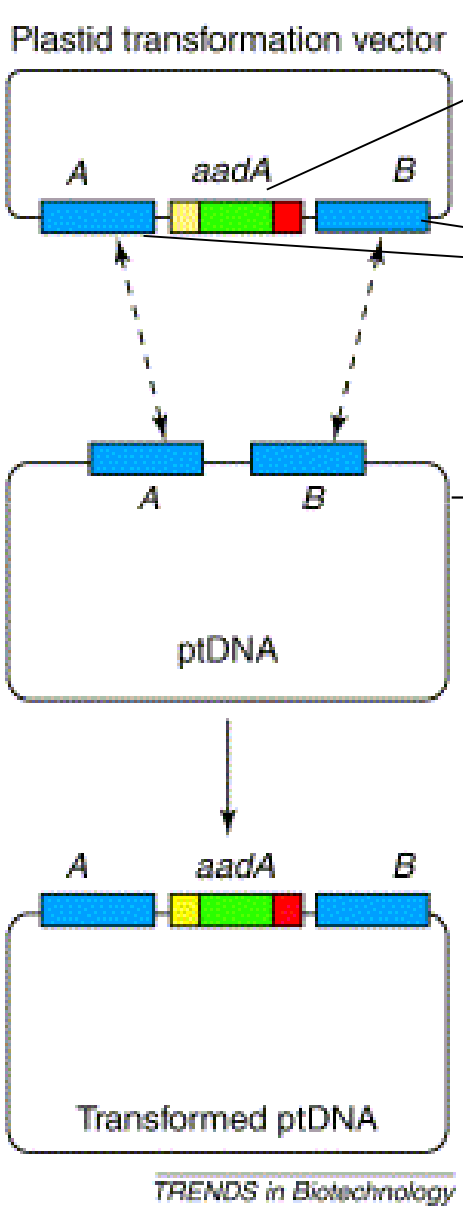
- Purification facilitée

- Pas de transmission par le pollen (L’ADN chloroplastique est transmis par les gamétes femelles)

2a. Techniques d'expression chez les plantes

Technique de transformation du chloroplaste





Gène d'intérêt

Séquences d'insertion (identiques aux régions d'intérêt de l'ADN chloroplastique)

ADN chloroplastique

Apres bombardement des feuilles avec l'ADN contenant le gène d' intérêt et la recombinaison homologue (insertion) avec l'ADN chloroplastique, on obtient une plante transformée.

Optimisation : modifications post-traductionnelles (Glycosylation, carboxylation, lipidation)

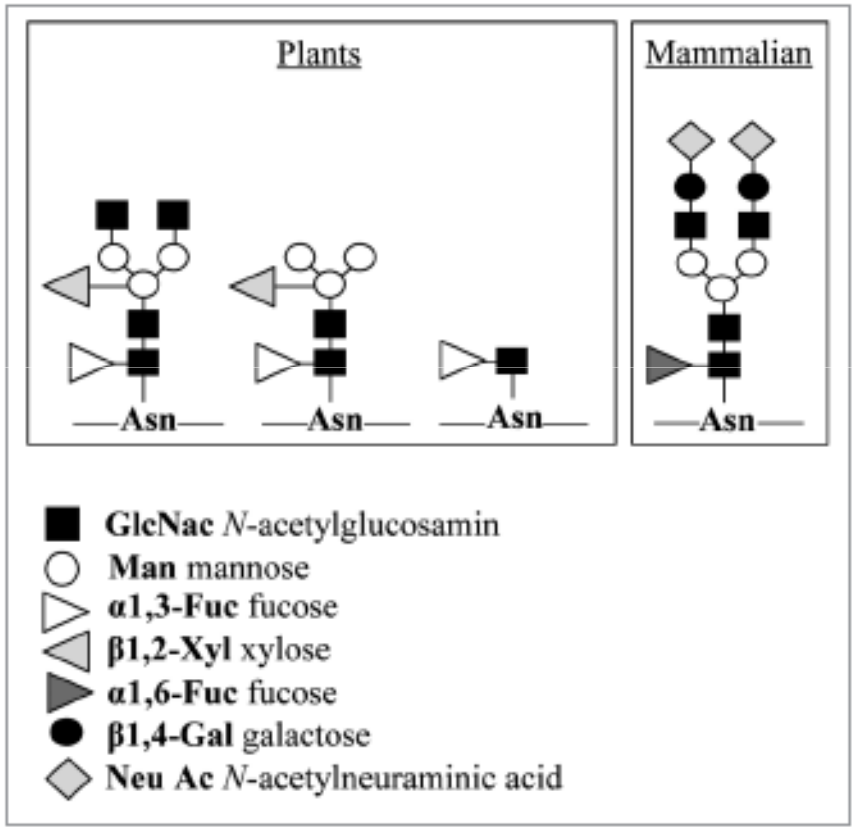


Figure 1. Typical N-glycan structures of recombinant glycoproteins produced in plant leaves (left), seed endosperm (middle and right) and mammalian cells (far right).

Peters and Stoger, 2011 (Human Vaccines)

2b. Systemes d'expression chez les plantes

Choix du system d'expression:

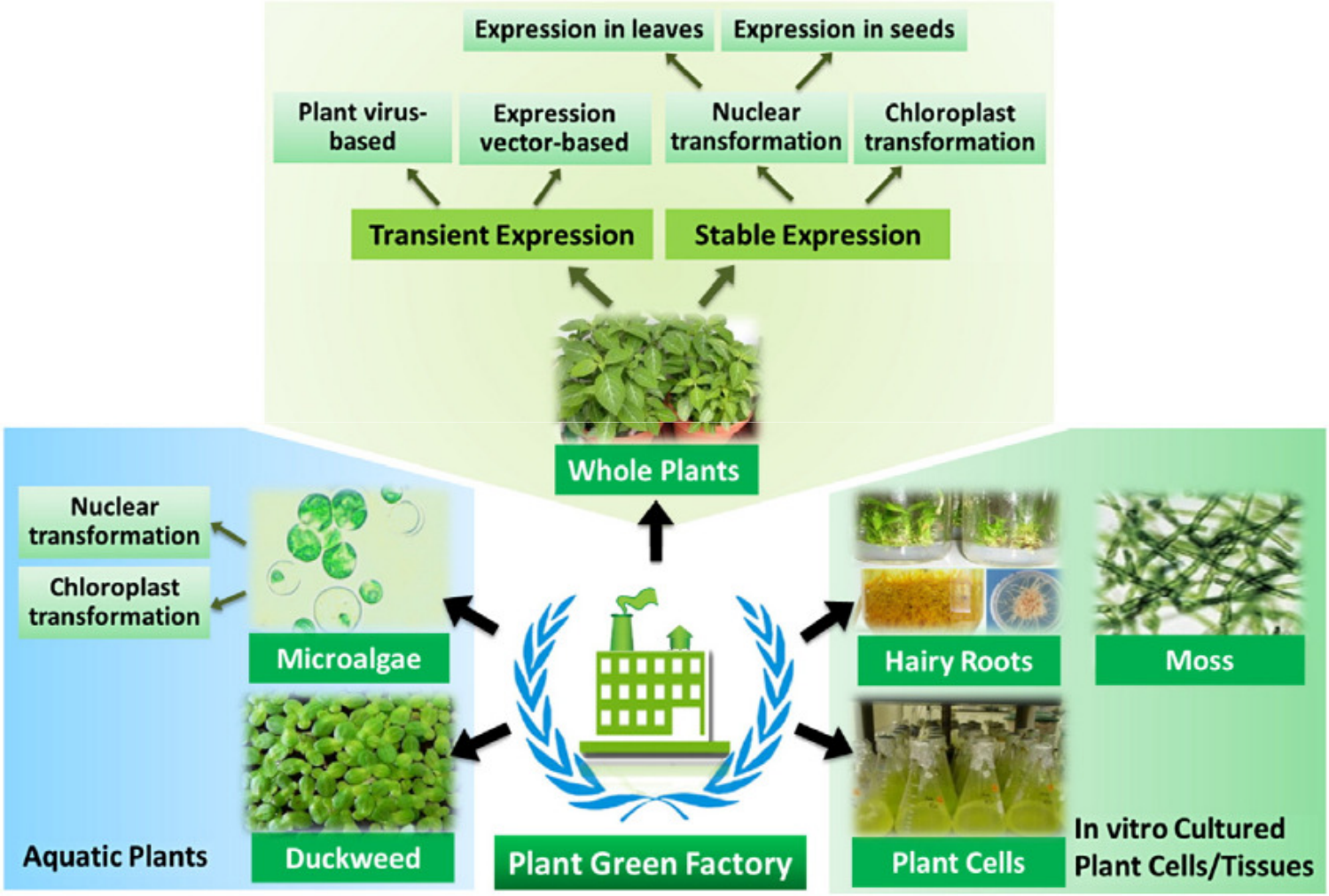


Fig. 1. Various plant cell expression platforms for the production of recombinant proteins.

Xu et al, 2012 (Biotechnology Advances 30)

Expression dans les feuilles:

> Tabac, luzerne (Alfalfa), soja, laitue…

Tobacco

- Technical Feasibility
  - Transformation ability routine
  - Protein production in leaf tissue; medium level of expression
  - Glycosylation occurs with nuclear transformation; no glycosylation with chloroplast transformation, reducing flexibility of protein production
  - Some IP issues
- Production Feasibility
  - Fair germplasm base available
  - Tobacco is a more expensive crop to grow
  - Purification more difficult with tissue-based production
  - More by-product with tissue based production
- Containment
  - Seed production typically prevented; chloroplast transformation reduces dissemination by seed
  - Minimum ¼ mile isolation distance
  - No wild relatives in most US planting areas
  - Seed dormancy in soil less than 2 years
  - Crop does not persist without intervention
- Environmental impact: Driven by specific protein
- Food/feed impact
  - Not a food or feed crop; non-target species unlikely to feed
  - Food safety generally not established
  - Risk driven by specific protein

BioTech Decisions Inc.

Expression dans les graines:

> cereales: Rice, maïs, blé; legumineuses: pois, soja

Rice

- Technical Feasibility
  - Transformation ability relatively routine
  - Stable protein storage in grain; high level of expression
  - Glycosylation occurs; high flexibility in protein production
  - Intellectual property issues relatively well understood
- Production Feasibility
  - Very good germplasm base available
  - Difficult to find US acres with food concerns
  - Good economics of production
  - Ease of purification good if targeted to endosperm
  - More limited by-product with grain
- Containment
  - Primarily self-fertilized
  - Relatively lower separation requirement
  - Presence of weedy red rice (relative) must be determined, mitigated and monitored
  - Seed dormancy in soil less than 2 years
  - Crop does not persist without intervention
- Environmental impact: Driven by specific protein
- Food/feed impact
  - Primarily a food crop
  - Rice itself not a common allergen, anti-nutritional or orally toxic
  - Risk driven by specific protein

BioTech Decisions Inc.

Expression dans les fruits/organes comestibles

> Production de protéines dans des organes particuliers

- Exemples: Fruits (bananes, tomates), pommes de terres)

- Facilités de récolte et de purification

> Production de vaccins oraux dans les plantes

- Ingestion d’antigènes produits par la plante
 -> immunisation

- Faible cout, facilement accessibles dans les pays en voie de développent

2b. Systemes d'expression chez les plantes

Celllules de plantes, mousses, et algues vertes:

Biotechnol Lett (2010) 32:1373–1383
DOI 10.1007/s10529-010-0326-5

REVIEW

Micro-algae come of age as a platform for recombinant protein production

Elizabeth Specht · Shigeki Miyake-Stoner · Stephen Mayfield

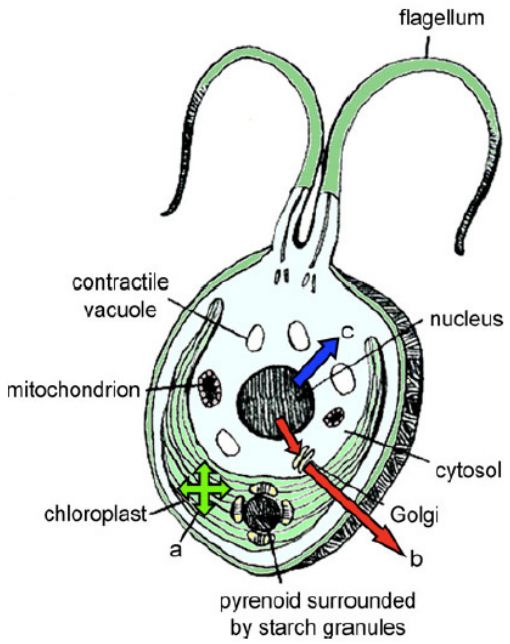


Fig. 1 *Chlamydomonas reinhardtii* as a versatile recombinant protein production platform. Protein expressed from the chloroplast genome is accumulated inside the single large chloroplast (*a*). The reducing environment of the chloroplast allows for proper folding of heavily disulfide bonded proteins, which is not easily accomplished in bacterial production platforms. Protein expressed from the nuclear genome accumulates in the cytosol (*b*), unless it is given an export signal sequence. In this case, it is sent to the endoplasmic reticulum for translocation and processing and then moves to the Golgi apparatus for packaging and export to the extracellular media (*c*)

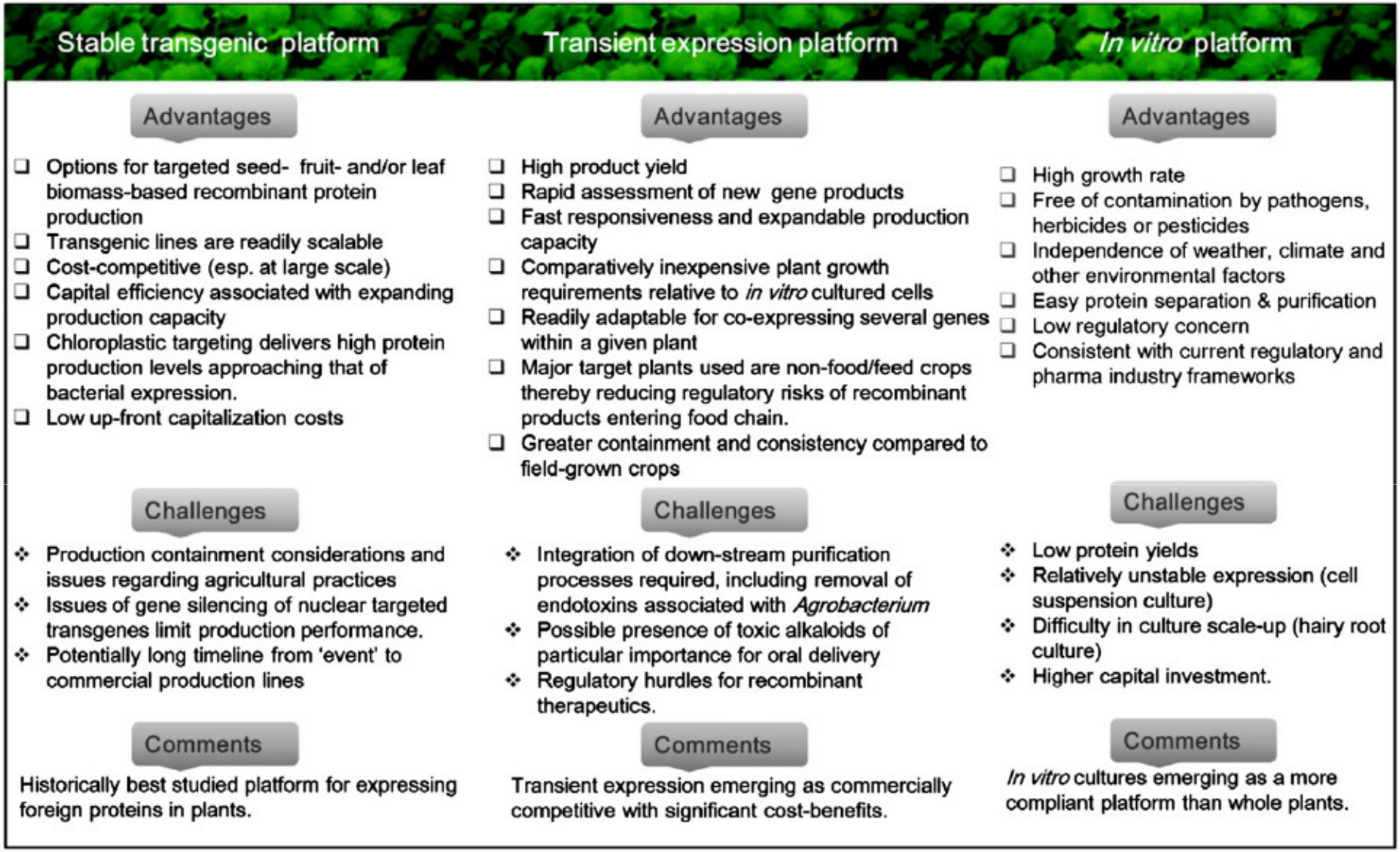


Fig. 2. Comparison of benefits and challenges associated with the three major types of plant production platforms. Because they are common to all plant platforms, the advantages of plants over other production systems, e.g., eukaryotic protein processing, lack of human pathogens, scalability options, are not listed here.

Xu et al, 2012 (Biotechnology Advances 30)

Table 3
Comparisons of different expression systems.

Expression system	Commercially viable species	Time for production[a]	Scalability	Regulatory compliance
Whole plants				
• Stable transgenic plants	Corn, soy, safflower, rice	3–6 months	Unlimited field culture	Difficult
• Transient plants	*Nicotiana* sp., lettuce	2–7 days	Greenhouse limited	Moderate
In vitro cultured plant cells and species				
• Hairy roots	*Nicotiana* sp.	14–30 days	20,000 L	Easy
• Cell suspension culture	Tobacco BY-2, carrot, rice	7–20 days	100,000 L	Easy
• Moss	*Physcomitrella patens*	14–30 days	200 L	Easy
Aquatic plants				
• Duckweed (closed system)	*Lemna* sp., *spirodela* sp.	20–40 days	10,000 L	Moderate
• Microalgae	*Chlamydomonas reinhardtii*			
Open system		20–40 days	Limited by water surface area	Difficult
Photobioreactor		14–30 days	10,000 L	Moderate
Conventional bioreactor		7–20 days	200 L	Easy

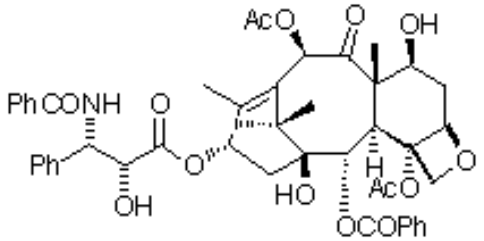
[a] The time required to accumulate maximum amounts of recombinant proteins in a culture system after planting or bioreactor inoculation.

Xu et al, 2012 (Biotechnology Advances 30)

3. Production de Métabolites

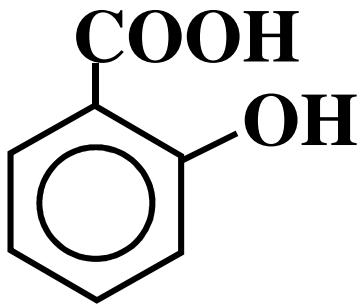
3. Production de Métabolites

If



Taxol
(antitumoral)

Saule



Acide Salicylique
(Aspirine)

25% of all commonly prescribed pharmaceuticals are directly or indirectly (via semisynthesis) derived from plants, (examples: paclitaxel, campthothecin, vincristine, morphine, codeine, and steroidal hormones), representing a market value of over 40 billion € in western countries

Production de substances médicalement actives ingérées avec la plante

Example: le riz doré (golden rice)

-Carence en vitamineA (dérivé du caroténe) chronique dans de nombreux pays
- +100 millions d'enfants -> cécité, maladies infantiles

- Production de provitamine A

- Transformation du riz avec gènes de biosynthése des caroténoïdes exprimés dans le grain

- prometteur mais production encore insuffisante (100 µg de Vit A pour 300 g de riz)

Modifications métaboliques des plantes ornementales

Modification du métabolisme des anthocyanes par transfert du géne de la Dihydro Flavonol 3 Reductase (DFR) et de la flavonoid 3'5' hydrolase de pétunia

Florigene Moonaqua™

Florigene Moonlite™

Florigene Moonvista™

FLORIGENE

FLOWERS

Smart ideas, better flowers

Pour approfondir…

- Twyman et al, 2003 (Trends in Biotechnology)
- Fischer et al, 2004 (Current Opinion in Plant Biology)
- Peters and Stoger, 2011 (Human Vaccines)
- Wilson and Roberts, 2012 (Plant Biotechnology journal)
- Obembe et al, 2011 (Biotechnology Advances)
- Special issue 2012 of Biotechnology Advances (30)

Molecular Farming: Plant-Made Pharmaceuticals and Technical Proteins

Editor(s): Rainer Fischer, Stefan Schillberg

Published Online: 6 JUN 2005

Print ISBN: 9783527307869

Online ISBN: 9783527603633

DOI: 10.1002/3527603638

Comparatif des systèmes d'expression

Expressions System	Yeast	Bacteria	Plant viruses	Transgenic Plants	Animal Cell Cultures	Transgenic Animals
Cost of maintaining	inexpensive	inexpensive	inexpensive	inexpensive	expensive	expensive
Type of storage	-2.0°C	-2.0°C	-2.0°C	RT*	N_2**	N/A
Gene size (protein) restriction	Unknown	Unknown	Limited	Not limited	Limited	Limited
Production cost	Medium	Medium	Low	Low	High	High
Protein yield	High	Medium	Very high	High	Medium to high	High
Therapeutic risk	Unknown	yes	Unknown	Unknown	yes	yes

Comparatif des systèmes d'expression

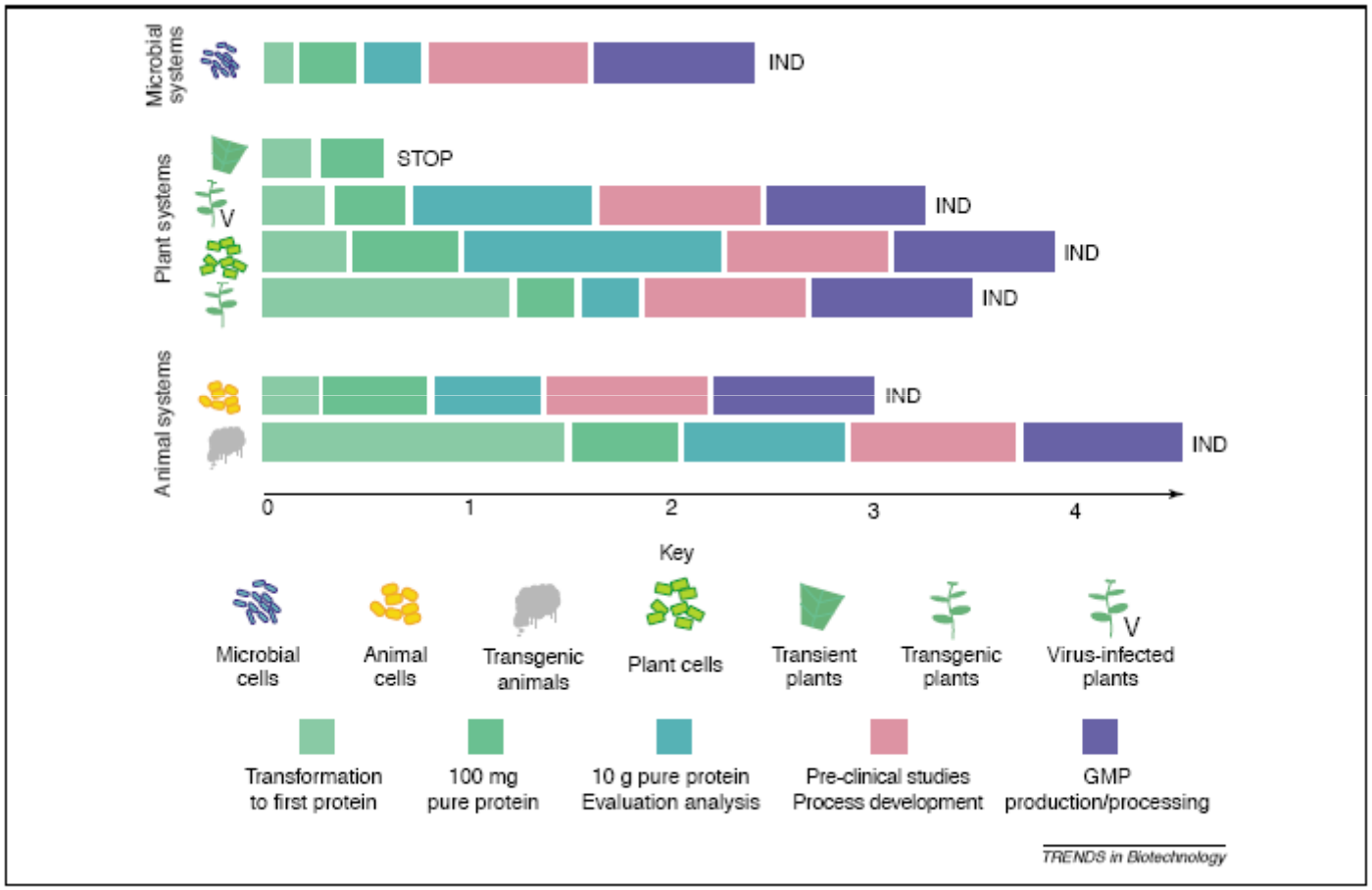


Figure 1. Performance of plant-based production systems in comparison to other commercial platforms for the production of recombinant proteins. Abbreviations: IND, investigational new drug.

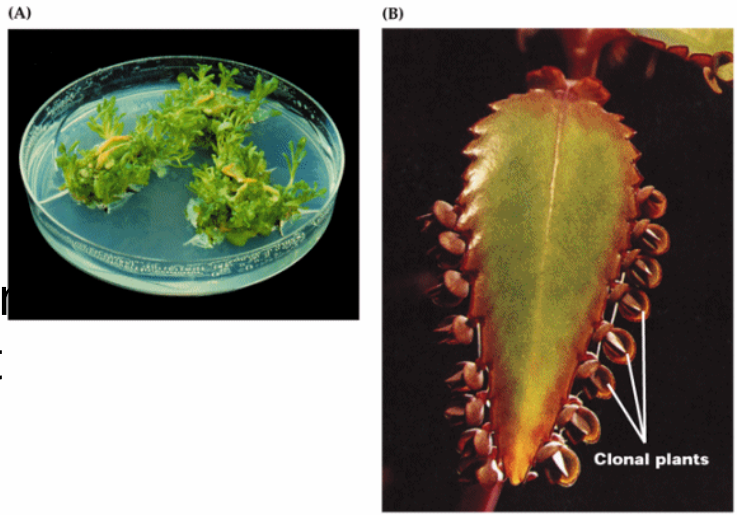
Twyman et al, 2003 (Trends in Biotechnology)

Probléme: comment introduire un gène nouveau dans toutes les cellules d'un organisme multicellulaire???

- La plupart des cellules végétales peuvent se développer en plantes entiéres
- Soit en formant des embryons
- Soit en formant des bourgeons
- Cette propriété n'est pas un artefact de culture, mais existe dans la nature (boutures)

Totipotence

->Si on peut transformer génétiquement une cellule on peut espérer regénérer une plante entiére
-> Il faut maîtriser les techniques de culture et les conditions de regénération a partir de tissus des plantes que l'on veut transformer



Exemple: transformation génétique du tabac

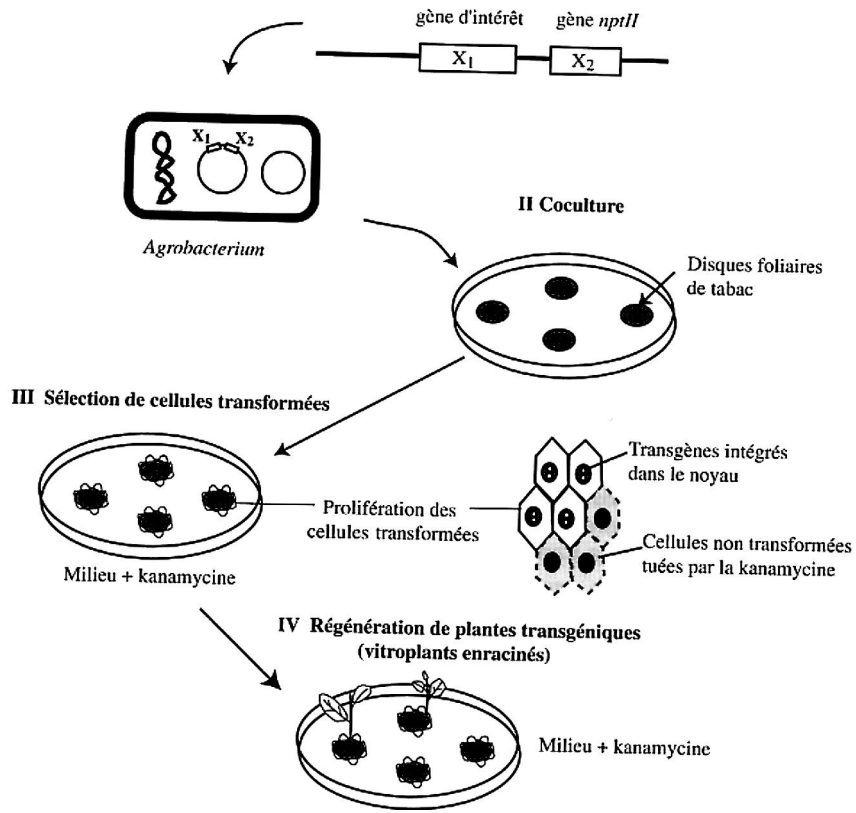


Figure 1. Les étapes de la transformation génétique du tabac.
I : le vecteur de transformation est introduit dans une souche d'*Agrobacterium tumefaciens* ; **II** : le transfert génétique a lieu au moment de la coculture ; **III** : sélection des cellules transformées ; **IV** : phase de régénération des plantes ; **V** : l'expérimentation au champ et la commercialisation des plantes ne sont pas figurées. X_1 : gène d'intérêt = gène de résistance à un insecte ; X_2 : gène *nptII* conférant une résistance à la kanamycine.

Plus facile: transformation génétique d'arabidopsis

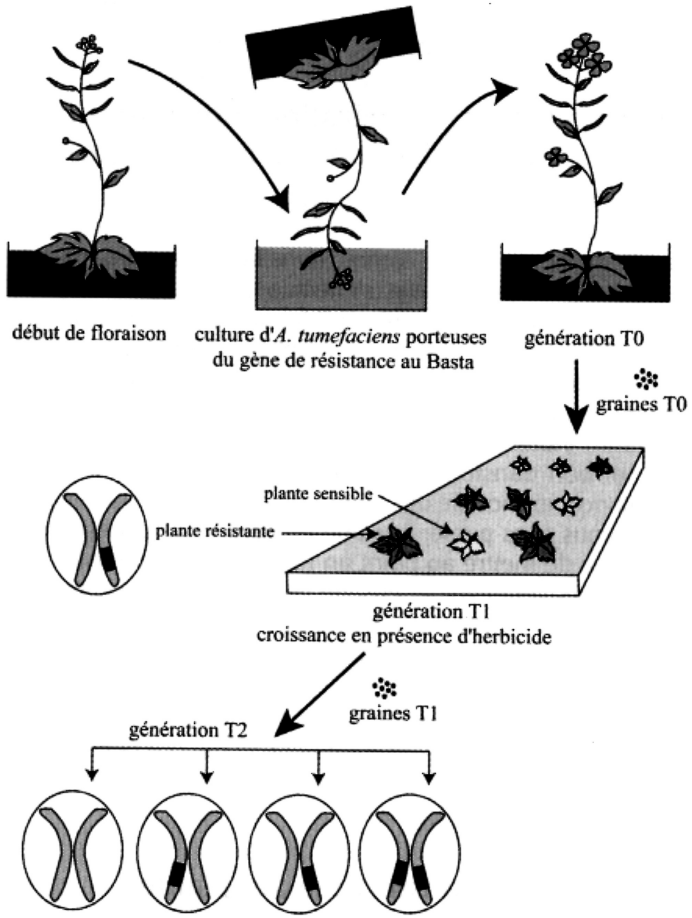


Figure 4. Transformation génétique d'*Arabidopsis thaliana* par infiltration.
Les plants d'*Arabidopsis* sont cultivés en serre ou en phytotron. Dès que leurs inflorescences présentent de nombreux bourgeons floraux encore immatures, elles sont trempées pendant 5 s dans une solution contenant du saccharose, un agent tensio-actif (Silwet) et la souche d'*Agrobacterium* utilisée pour la transformation. Cette souche porte dans un vecteur de transformation binaire les gènes d'intérêt et un gène de sélection conférant la résistance à un herbicide, le Basta. Les graines sont ensuite récoltées, et sélectionnées après germination en présence de l'herbicide. Les plantes résistantes de la génération T1 étant hémizygotes, les transgènes intégrés dans l'un de leurs chromosomes se retrouvent, après méiose, dans la moitié des gamètes produits ; après fécondation, on obtient donc un quart de plantes homozygotes dans la génération T2.

Les agrobactéries hébergent de très grands plasmides nécessaires à La formation des tumeurs/chevelu racinaires

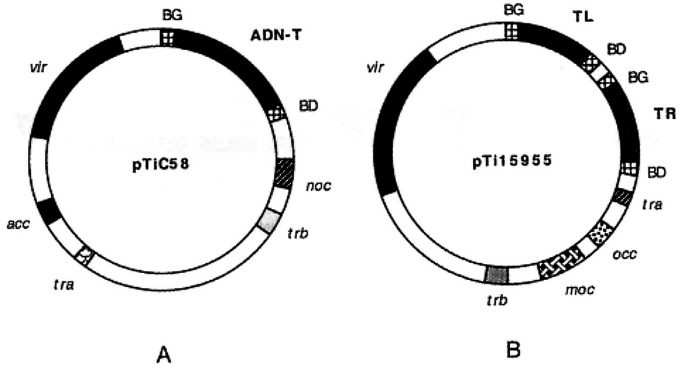
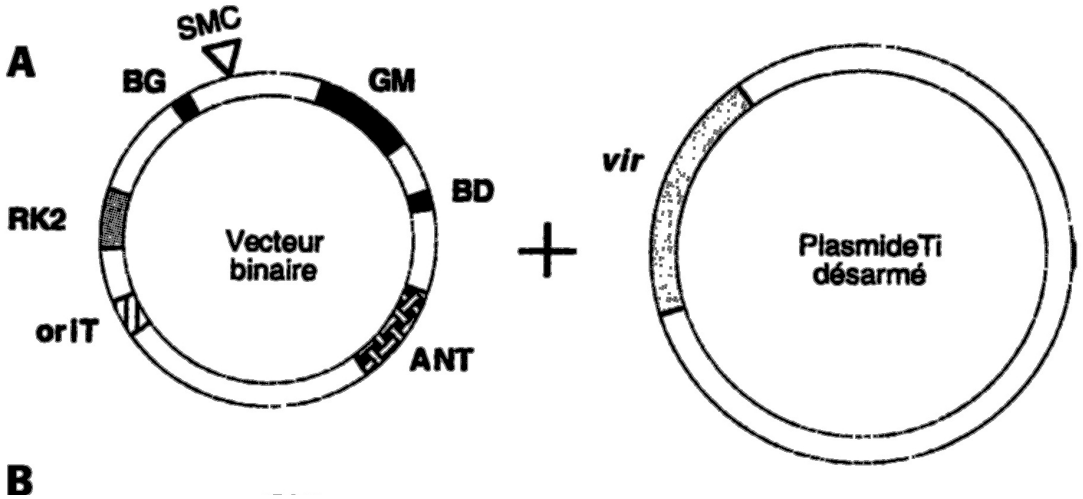


Figure 3. Organisation générale des plasmides Ti à octopine **(A)** et à nopaline **(B)**.
L'ADN-T est encadré des zones de bordure : BG, bordure gauche et BD, bordure droite (plasmide pTiC58). Le plasmide à nopaline (pTi15955) possède deux parties distinctes dans l'ADN-T, une partie gauche (TL) et une partie droite (TR) qui sont transférées de manière indépendante dans la cellule végétale. *vir* : région de virulence ; *noc, acc,* et *occ* : régions portant le catabolisme de la nopaline, de l'agrocinopine et de l'octopine ; *tra* et *trb* : régions portant les fonctions de conjugaison.

A. Tumefaciens -> plasmide Ti

Nopaline BG	GGCTGGCTGG	TGGCAGGATATATTGTGGTGTAAA	CAAATT
Nopaline BD	TATCAGTGTT	TGACAGGATATATTGGCGGGTAAA	CCTAAG
Octopine ADN-TL BG	GCGGCAGCGG	CGGCAGGATATATTCAATTGTAAA	ATGGCT
Octopine ADN-TL BD	TGATGCTGAC	TGGCAGGATATATACCGTTGTAAT	TTTGAG
Octopine ADN-TR BG	TGAGAAAAGG	TGGCAGGATATATCGAGGTGTAAA	ATATCA
Octopine ADN-TR BD	TGATGACTGA	TGGCAGGATATATGCGGTTGTAAT	TCATTT
Séquence consensus Ti	TG------G-	TGGCAGGATATAT---G-TGTAAA	------

Modification des plasmides Ti pour transférer des gènes
-délétion des gènes responsables de la croissance tumorale
-Délétion des génes de synthése d'opine
-Addition d'un gène sélectionnable (résistance aux antibiotiques ou herbicides)
- Séparation en deux plasmides -> système binaire



Production:de l'ordre du gramme de protéines recombinante
par kg de tissus inoculé

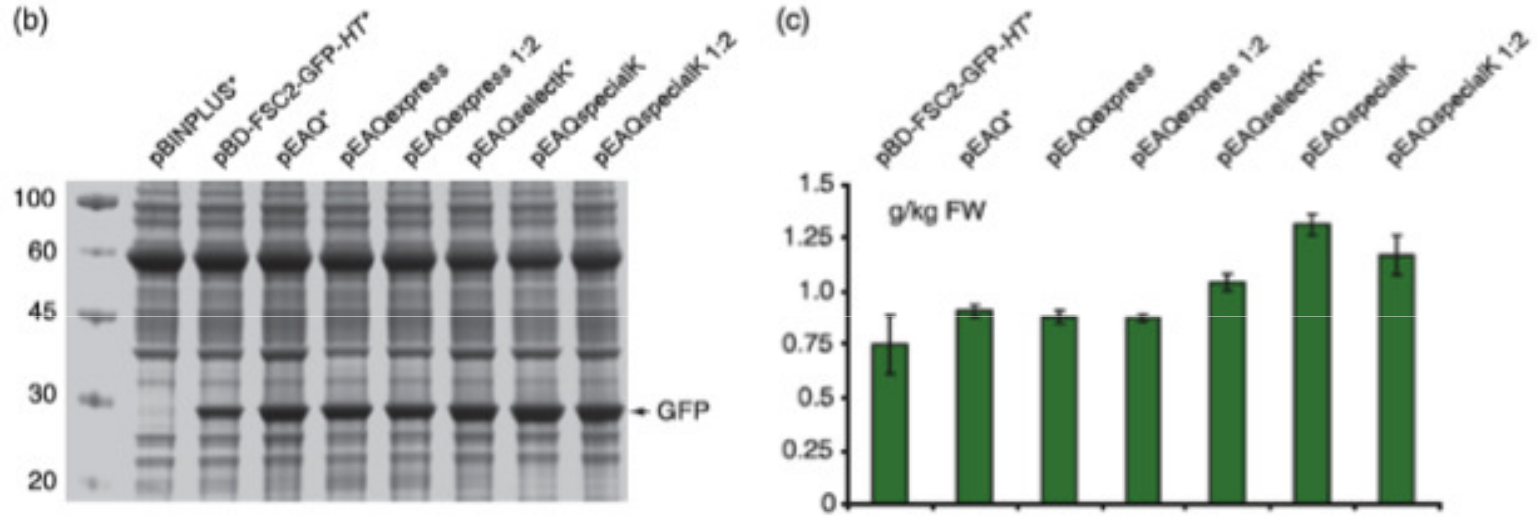


Figure 3 Expression levels from pEAQ and its derivatives compared with the parent plasmid pBINPLUS. 1 : 2 = constructs infiltrated at half the standard OD600; and * = constructs co-infiltrated with P19. (a) Leaves visualized under UV light, (b) Coomassie-stained 12% SDS-PAGE with molecular weight size markers indicated, and (c) spectrofluorometric analysis of GFP expression where values represent 6 samples from 2 separate experiments ± SE.

Purification des protéines par chromatographie d'affinité

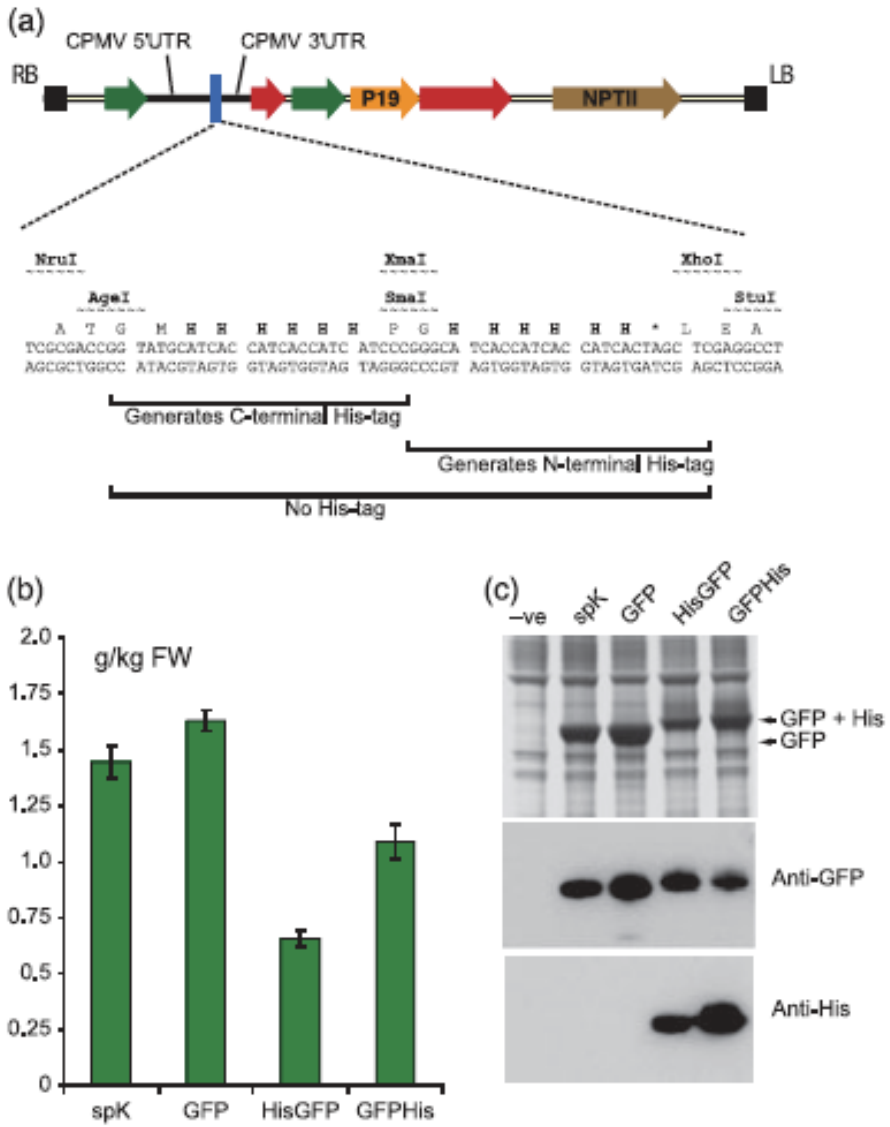


Figure 5 pEAQ-*HT* cloning and expression of GFP as native and His-tagged variants. (a) Diagrammatic representation of the T-DNA region of pEAQ-*HT* showing the polylinker in detail. Black boxes, T-DNA borders; green arrows, promoter sequences; and red arrows, terminator sequences. (b) Spectrofluorometric analysis of GFP expression. spK = pEAQspecialK-GFP-*HT* and GFP, HisGFP, and GFPHis are the pEAQ-*HT* clones. (c) 12% SDS-PAGE and Western blot analysis of GFP variant expression; –ve, control extract.

OPEN ACCESS Freely available online

PLoS one

Rapid Transient Production in Plants by Replicating and Non-Replicating Vectors Yields High Quality Functional Anti-HIV Antibody

Frank Sainsbury[1*¤a], Markus Sack[2], Johannes Stadlmann[3], Heribert Quendler[4¤b], Rainer Fischer[2,5], George P. Lomonossoff[1]

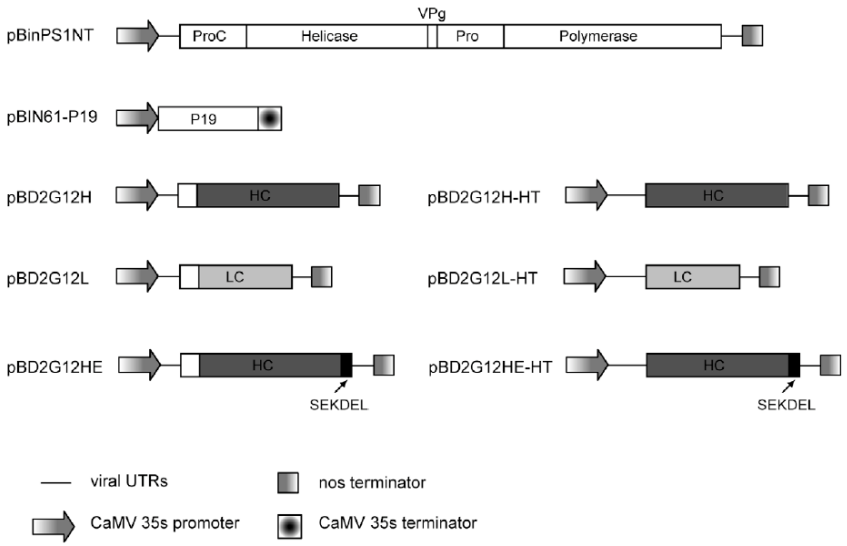


Table 1. Purification of ^{CPMV}2G12 and ^{HT}2G12 variants from infiltrated tissue.

2G12 variant	Leaf mass (g)	Extract volume (ml)	2G12 conc. (μg/ml)	2G12 yield (mg)	% Recovery	mg recovered/kg of fresh weight tissue
^{CPMV}2G12HL	176.0	485	5.37	2.6	73%	10.8
^{HT}2G12HL	134.9	435	20.57	9.0	79%	52.6
^{CPMV}2G12HEL	105.9	315	12.75	4.0	71%	26.9
^{HT}2G12HEL	85.6	290	36.56	10.6	85%	105.1

ELSEVIER

Vaccine 23 (2005) 1875–1880

www.elsevier.com/locate/vaccine

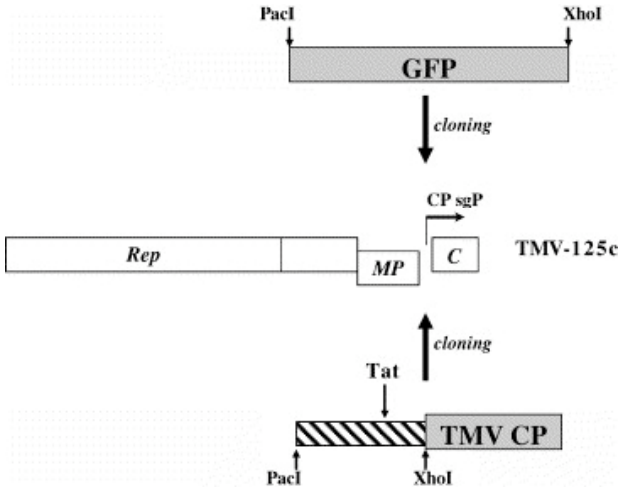
Plant based HIV-1 vaccine candidate: Tat protein produced in spinach

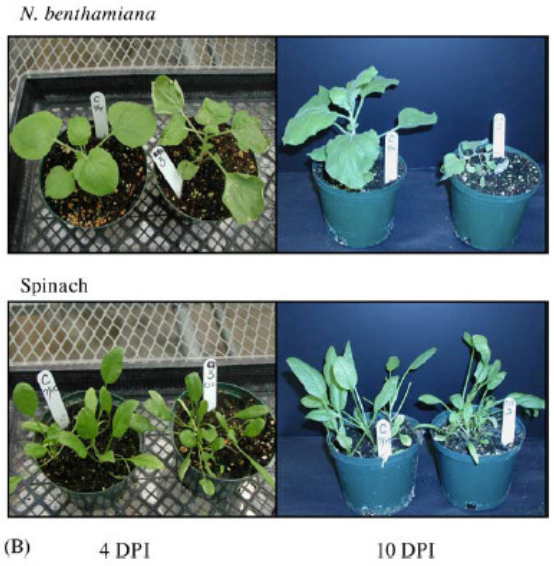
Alexander V. Karasev[a,*], Scott Foulke[b], Candice Wellens[a], Amy Rich[a], Kyu J. Shon[a], Izabela Zwierzynski[a], David Hone[b], Hilary Koprowski[a], Marvin Reitz[b]

[a] *Biotechnology Foundation Laboratories at Thomas Jefferson University, Department of Microbiology and Immunology, 700 E. Butler Avenue, Doylestown, PA 18901, USA*

[b] *Institute of Human Virology, University of Maryland Biotechnology Institute, Baltimore, MD 21202, USA*

Available online 19 November 2004





Utilisation d'un vecteur viral réplicatif

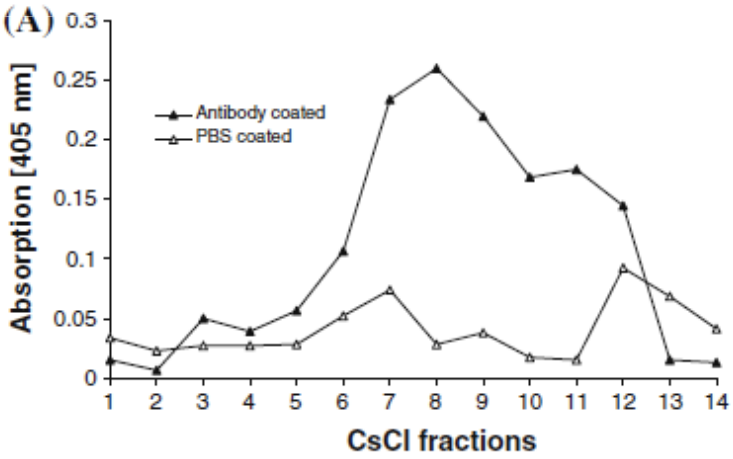
Table 1
Immunoreactivity of the plant-produced Tat in a Western-blot assay tested against a panel of available Tat-specific monoclonal antibodies

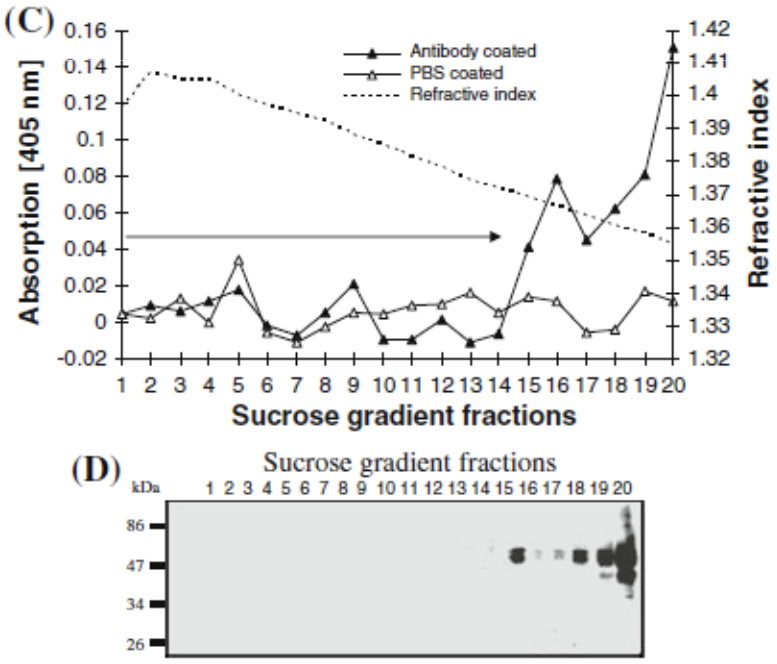
MAb	Original antigen	Reactivity with *Tat*CP
ABI #161	aa 1–16	++
#15.1	aa 1–16	+/–
#4138	aa 1–15	++
NT8 8D1.8	Whole tat	++
NT7 7D5.1	Whole tat	–
NT7 4A4.8	Whole tat	+/–

Transgenic Res (2011) 20:271–282
DOI 10.1007/s11248-010-9415-4

ORIGINAL PAPER

Transplastomic expression of a modified human papillomavirus L1 protein leading to the assembly of capsomeres in tobacco: a step towards cost-effective second-generation vaccines





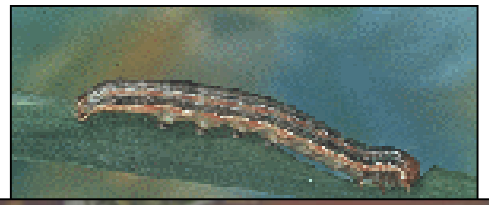
Quelques applications de la transformation du chloroplaste

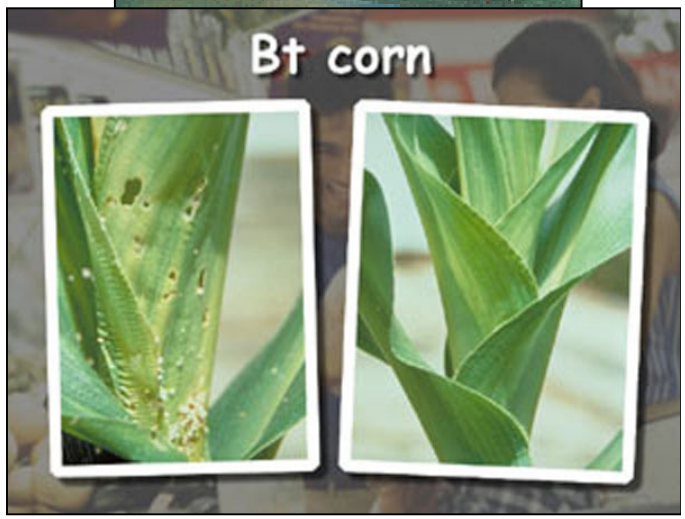
La résistance au glyphosate

Le Glyphosate est un puissant herbicide avec un bas impact environnemental, utile pour éliminer les plantes infestantes d'une culture résistante.
Daniell et al. (1998) a transformé avec succès des plantes de tabac avec un gène de résistance au glyphosate inséré dans le génome du chloroplaste. Les plantes sont résistantes et le gène ne peut pas être transféré par le pollen à des autre plantes.

Résistance aux insectes grâce à la toxine Bt

Les toxines du Bacillus thuringensis (Bt) sont toxiques pour les insectes après ingestion (mais elles ne sont pas toxiques pour les animaux). Kota et al. (1999) ont vu que l'expression de la toxine Bt dans les chloroplastes de plante porte à une mortalité élevée des insecte en protégeant les plantes des attaques. En plus l'expression de la toxine est localisée dans les feuilles et absente dans le tissus (fruits, grains) qui sont mangés par les animaux.





Optimisation : Glycosylation

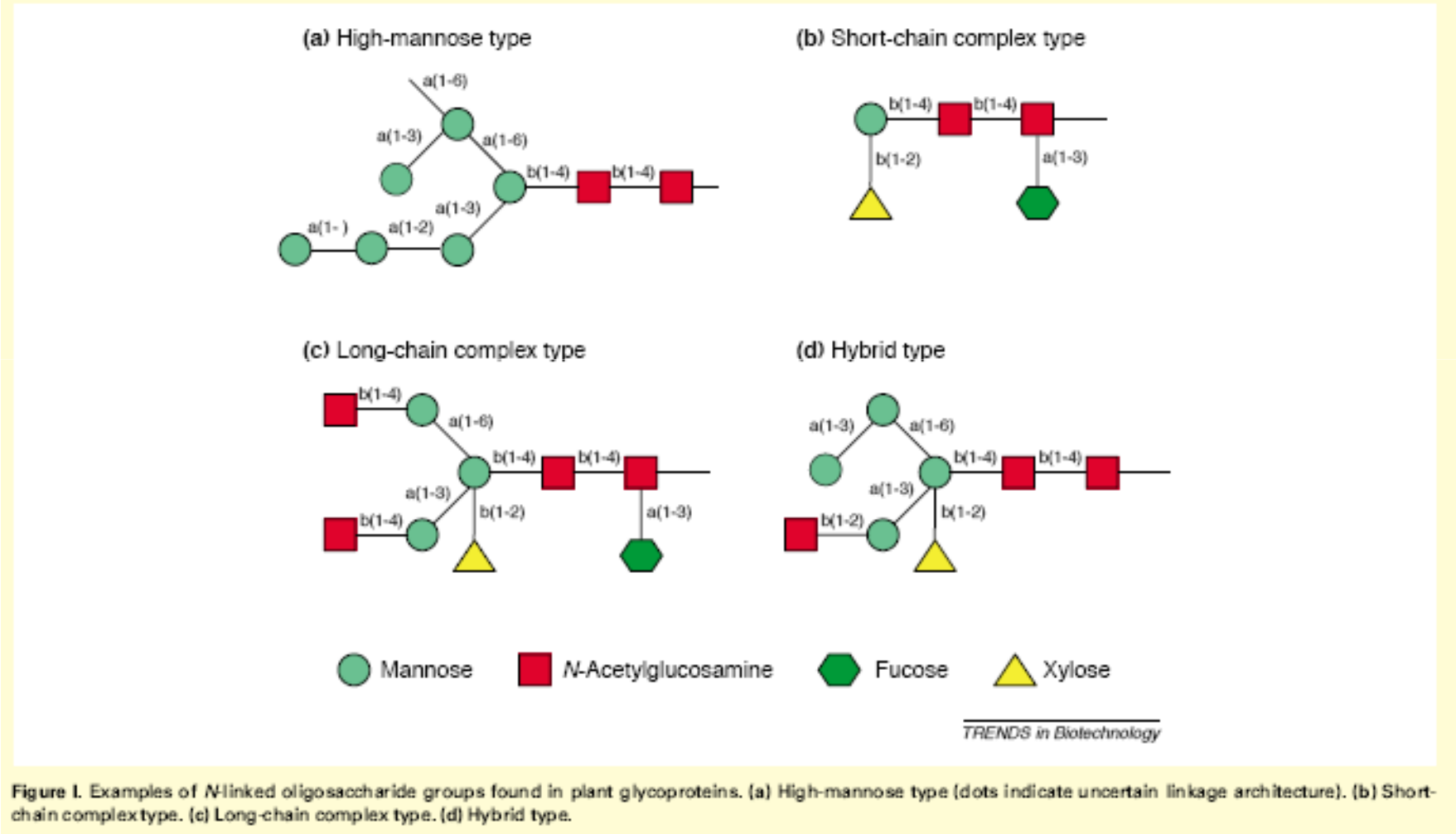
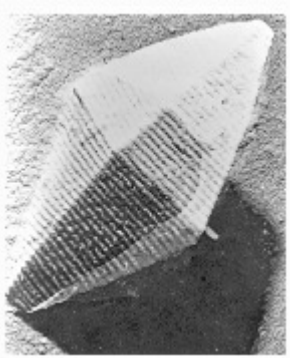
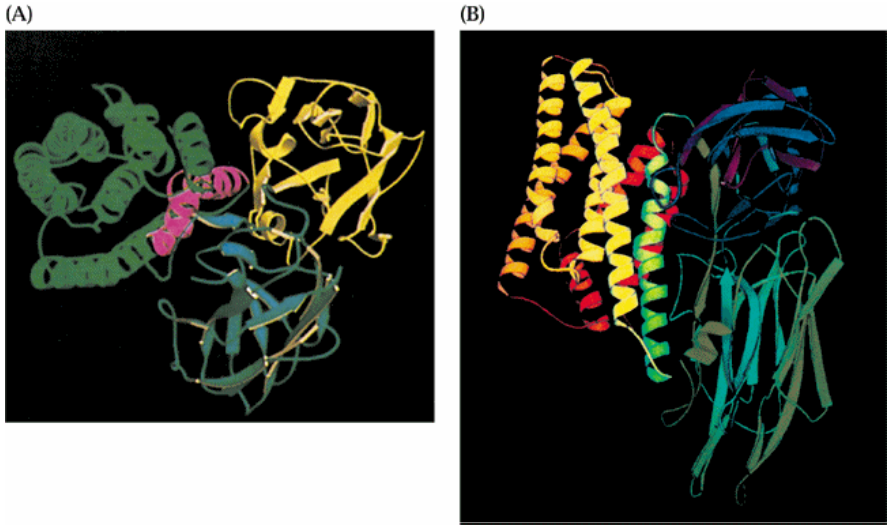


Figure I. Examples of *N*-linked oligosaccharide groups found in plant glycoproteins. (a) High-mannose type (dots indicate uncertain linkage architecture). (b) Short-chain complex type. (c) Long-chain complex type. (d) Hybrid type.

Twyman et al, 2003 (Trends in Biotechnology)

La production de nouvelles protéines a aussi un interêt en amélioration des plantes





Endotoxine de Bacillus thuriengensis (Bt)
-protoxine clivée dans l'intestin moyen de l'insecte par protéase + pH alcalin
-se fixe a des sites de l'intestin
-ulceration, perte d'appétit
-Rupture des cellules, mort des larves

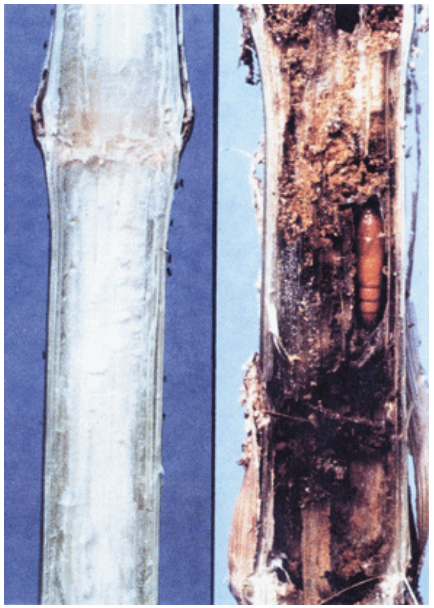


Table 1 Recent successes in therapeutic protein production in algae

Gene expressed	Function	Expression level achieved	Application	Source
HSV8-lic	First mammalian protein expressed, antibody	Detectable	Pharmaceutical	Mayfield et al. (2003)
CTB-VP1	Cholera toxin B subunit fused to foot and mouth disease VP1	3% TSP	Vaccine	Sun et al. (2003)
HSV8-scFv	Classic single-chain antibody	0.5% TSP	Pharmaceutical	Mayfield et al. (2005)
hMT-2	Human metallothionine-2	Detectable	Pharmaceutical, UV-protection	Zhang et al. (2006)
hTRAIL	Human tumor necrosis factor-related apoptosis-inducing ligand (TRAIL)	~0.67% TSP	Pharmaceutical	Yang et al. (2006)
M-SAA	Bovine mammary-associated serum amyloid	~5% TSP	Therapeutics, oral delivery	Mamaell et al. (2007)
CSFV-E2	Swine fever virus E2 viral protein	~2% TSP	Vaccine	He et al. (2007)
hGAD65	Diabetes-associated anutoantigen human glutamic acid decarboxylase 65	~0.3% TSP	Diagnostics and therapeutics	Wang et al. (2008)
ARS2-crEpo-his6	Human erythropoietin fused to ARS2 export sequence w/6xhis tag	100 μg/l culture	Pharmaceutical, protein export	Eichler-Stahlberg et al. (2009)
83K7C	Full-length IgG1 human monoclonal antibody against anthrax protective antigen 83	0.01% dry algal biomass	Therapeutics	Tran et al. (2009)
IgG1	Murine and human antibodies (LC and HC)	Detectable	Therapeutics	Tran et al. (2009)
VP28	White spot syndrome virus protein 28	~10.5% TSP	Vaccine	Surzycki et al. (2009)
CTB-D2	D2 fibronectin-binding domain of Staphylococcus aureus fused with the cholera toxin B subunit	0.7% TSP	Oral vaccine	Dreesen et al. (2010)
10NF3, 14FN3	Domains 10 and 14 of human fibronectin, potential antibody mimics	14FN3: 3% TSP 10FN3: detectable	Therapeutics	Rasala et al. (2010)
M-SAA-Interferon β1	Multiple sclerosis treatment fused to M-SAA	Detectable	Therapeutics	Rasala et al. (2010)
Proinsulin	Blood sugar level-regulating hormone, type I diabetes treatment	Detectable	Therapeutics	Rasala et al. (2010)
VEGF	Human vascular endothelial growth factor isoform 121	2% TSP	Therapeutics	Rasala et al. (2010)
HMGB1	High mobility group protein B1	2.5% TSP	Therapeutics	Rasala et al. (2010)

Table 2. Selected pharmaceutical proteins expressed in transgenic plants

Year	Protein	Transformed species	Reference
1986	Human growth hormone	*N. tabacum* *H. annus*	(Barta et al., 1986)
1990	Human serum albumin	*N. tabacum* *S. tuberosum*	(Sijmons et al., 1990)
1993	Human epidermal growth factor	*N. tabacum*	(Higo et al., 1993)
1994	Trout growth factor	*N. tabacum*	(Bosch et al., 1994)
1994	Human α-interferon	*O. sativa*	(Zhu et al., 1994)
1995	Hirudin	*N. tabacum* Suspension cells	(Parmenter et al., 1995)
1995	Erythropoetin	*N. tabacum* Suspension cells	(Matsumoto et al., 1995)
1996	Glucocerebrosidase, human protein C serum protease	*N. tabacum*	(Cramer et al., 1996)
1997	Human α and β haemoglobin	*N. tabacum*	(Dieryck et al., 1997)
1997	Human muscarinic cholinergic receptors	*N. tabacum*	(Mu et al., 1997)
1997	Murine granulocyte-macrophage colony stimulating factor	*N. tabacum*	(Lee et al., 1997)
1998	Interleukin-2 and Interleukin-4	*N. tabacum* Suspension cells	(Magnuson et al., 1998)
1999	Human placental alkaline phosphatase	*N. tabacum* Rhizosecretion	(Borisjuk et al., 1999)
1999	Human α1-antitrypsin	*O. sativa* Suspension cells	(Terashima et al., 1999)
2000	Human growth hormone (somatotrophin)	*N. tabacum* seeds	(Leite et al., 2000)
2000	Human growth hormone (somatotrophin)	*N. tabacum* Chloroplasts	(Staub et al., 2000)

Fischer & Emans, 2000 (Transgenic Research)

Table 3. Recombinant antibodies expressed in transgenic plants

Year	Antibody format	Antigen	Plant organ	Cellular location	Transformed species	Reference
1989	IgG1	Phosphonate ester	Leaf	ER	*N. tabacum*	(Hiatt et al., 1989)
1990	IgM	NP hapten	Leaf	ER chloroplast	*N. tabacum*	(Düring et al., 1990)
1991	V_H domain	Substance P (neuropeptide)	Leaf	Intra- and extra-cellular	*N. benthamiana*	(Benvenuto et al., 1991)
1992	scFv	Phytochrome	Leaf	Cytosol	*N. tabacum*	(Owen et al., 1992)
1993	IgG1 Fab	Human creatine kinase	Leaf	Nucleolus	*N. tabacum* *A. thaliana*	(De Neve et al., 1993)
1993	scFv	Phytochrome	Leaf	Apoplast	*N. tabacum*	(Firek et al., 1993a)
1993	scFv	AMCV	Leaf	Cytosol	*N. benthamiana*	(Tavladoraki et al., 1993)
1994	IgG	Fungal cutinase	Root	Apoplast	*N. tabacum*	(van Engelen et al., 1994)
1994	IgG1	*Streptococcus mutans* adhesin	Leaf	Apoplast	*N. tabacum*	(Ma et al., 1994)
1995	IgA/G	*Streptococcus mutans* adhesin	Leaf	Apoplast	*N. tabacum*	(Ma et al., 1995)
1995	IgG	TMV	Leaf	Apoplast	*N. tabacum*	(Voss et al., 1995)
1996	scFv	Cutinase	Leaf	ER	*N. tabacum*	(Schouten et al., 1996)
1996	IgM	RKN secretion	Leaf root	Apoplast	*N. tabacum*	(Baum et al., 1996)
1996	scFv	BNYVV	Leaf	Apoplast	*N. benthamiana*	(Fecker et al., 1996)
1996	scFv	Human creatine kinase	Leaf	Cytoplasm ER	*N. tabacum*	(Bruyns et al., 1996)
1996	IgG1 Fab	Human creatine kinase	Leaf	Apoplast	*A. thaliana*	(De Wilde et al., 1996)
1997	scFv	β-1,4-endoglucanase	Root	Cytosol	*S. tuberosum*	(Schouten et al., 1997)
1997	scFv	Oxazolone	Leaf	ER	*N. tabacum*	(Fiedler et al., 1997)
1997	scFv	Abscisic acid	Leaf	ER	*N. tabacum*	(Fiedler et al., 1997)
1997	scFv	Abscisic acid	Seed	ER	*N. tabacum*	(Phillips et al., 1997)
1997	scFv-IT	CD-40	Plant	Apoplast	*N. tabacum* tissue culture	(Francisco et al., 1997)
1998	scFv	Oxazolone	tuber	ER	*S. tuberosum*	(Artsaenko et al., 1998)
1998	Humanized IgG1	HSV-2	Plant	Secretory pathway	*Glycine max*	(Zeitlin et al., 1998)
1998	scFv	Dihydro-flavonol 4-reductase	Leaf	Cytosol	*P. hybrida*	(De Jaeger et al., 1998)
1999	IgG	Human IgG	Plant	Apoplast	*Medicago sativa*	(Khoudi et al., 1999)
1999	scFv	CEA	Leaf	Transient expression	*N. tabacum*	(Vaquero et al., 1999)
1999	scFv	Tospoviruses	Plant	ER, apoplast	*N. benthamiana*	(Franconi et al., 1999)
1999	bi-scFv	TMV	Leaf	ER, apoplast	*N. tabacum* Suspension cells	(Fischer et al., 1999d)
1999	scFv	TMV	Plant	Cytosol	*N. tabacum*	(Zimmermann et al., 1998)
1999	scFv	CEA	Cell	ER, apoplast	*O. sativa* Suspension cells	(Torres et al., 1999)F
1999	scFv	38C13 mouse B cell lymphoma	Leaf	Apoplast	*N. benthamiana*	(McCormick et al., 1999)
2000	scFv	CEA	Plant	ER, apoplast	*O. sativa T. aestivum*	(Stöger et al., 2000)
2000	scFv	TMV	Leaf	Apoplast, membrane	*N. tabacum*	(Schillberg et al., in press)

CEA – carcinoembryonic antigen; ER – endoplasmic reticulum; AMCV – Artichoke mottle crinkle virus; TMV – tobacco mosaic virus; RKN – root knot nematode; BNYVV – beet nectrotic yellow vein virus; HSV-2 – herpes simplex virus-2; scFv-IT – scFv-bryodin-immunotoxin.

Fischer & Emans, 2000 (Transgenic Research)

Table 4. Recombinant vaccines expressed in plants

Year	Vaccine antigen	Transformed species	Reference
1992	Hepatitis virus B surface antigen	*N. tabacum*	(Mason et al., 1992)
1995	Malaria parasite antigen	Virus particle	(Turpen et al., 1995)
1995	Rabies virus glycoprotein	*L. esculentum*	(McGarvey et al., 1995)
1995	*Escherichia coli* heat-labile enterotoxin	*N. tabacum, S. tuberosum*	(Haq et al., 1995)
1996	Human rhinovirus 14 (HRV-14) and human immunodeficiency virus type (HIV-1) epitopes	Virus particle	(Porta et al., 1996)
1996	Norwalk virus capsid protein	*N. tabacum, S. tuberosum*	(Mason et al., 1996)
1997	Diabetes-associated autoantigen	*N. tabacum, S. tuberosum*	(Ma et al., 1997)
1997	Hepatitis B surface proteins	*S. tuberosum*	(Ehsani et al., 1997)
1997	Mink Enteritis Virus epitope	Virus particle	(Dalsgaard et al., 1997)
1997	Rabies and HIV epitopes	Virus particle	(Yusibov et al., 1997)
1998	Foot and mouth disease virus VP1 structural protein	*A. thaliana*	(Carrillo et al., 1998)
1998	*Escherichia coli* heat-labile enterotoxin	*S. tuberosum*	(Mason et al., 1998)
1998	*Escherichia coli* heat-labile enterotoxin	*S. tuberosum*	(Tacket et al., 1998)
1998	Rabies virus	Virus particle	(Modelska et al., 1998)
1998	Cholera toxin B subunit	*S. tuberosum*	(Arakawa et al., 1998a)
1998	Human insulin-Cholera toxin B subunit fusion protein	*S. tuberosum*	(Arakawa et al., 1998b)
1999	Foot and mouth disease virus VP1 structural protein	*Medicago sativa*	(Wigdorovitz et al., 1999)
1999	Hepatitis B virus surface antigen	*Lupinus luteus, Lactuca sativa*	(Kapusta et al., 1999)
1999	Human cytomegalovirus glycoprotein B	*N. tabacum*	(Tackaberry et al., 1999)
1999	Diabetes-associated autoantigen	*N. tabacum, D. carota*	(Porceddu et al., 1999)

Fischer & Emans, 2000 (Transgenic Research)

Table 1
Plant-derived pharmaceuticals in clinical stages of development or on market. Information from Basaran and Rodríguez-Cerezo (2008), Kaiser (2008), Key et al. (2008), Spök et al. (2008), Sharma and Sharma (2009), Lau and Sun (2009), Obembe (2010), Faye and Gomord (2010). Updated from company websites.

Product	Disease	Plant	Clinical trial status	Company	Source: URL/academic
Vaccines					
Hepatitis B antigen (HBsAg)	Hepatitis B	Lettuce Potato	Phase I Phase II	Thomas Jefferson University, USA Arizona State University	Streatfield, 2006
Fusion proteins, including epitopes from rabies	Rabies	Spinach	Phase I completed	Thomas Jefferson University, USA	www.labome.org/expert/usa/.../hilary-koprowski-233492.html
Cancer vaccine	Non-Hodgkin's lymphoma	Tobacco	Phase II	Large Scale Biology[a], USA	http://www.gmo-safety.eu/article/483.pharma-plants-status-report.html
Vibrio cholerae	Cholera	Potato	Phase I	Arizona State University	Tacket, 2005
Heat-labile toxin B subunit of *Escherichia coli*	Diarrhea	Maize Potato	Phase I Phase I	ProdiGene[b], USA Arizona State University	Tacket, 2005
Capsid protein Norwalk virus	Diarrhea	Potato, Tomato	Phase I	Arizona State University	Khalsa et al., 2004
Antigen	Feline parvovirus (Dogs)	Tobacco	Advanced	Large Scale Biology[a], USA	http://www.lsbc.com
Antigen	Papilloma virus (Rabbit)	Tobacco	Early	Large Scale Biology[a], USA	http://www.lsbc.com
HN protein of Newcastle disease virus	Newcastle disease (Poultry)	Tobacco suspension cells	USDA Approved	Dow Agro Sciences, USA	http://www.dowagro.com/animalhealth
Viral vaccine mixture	Diseases of horses, dogs, and birds	Tobacco suspension cells	Phase I	Dow Agro Sciences, USA	http://www.dowagro.com/animalhealth
Poultry vaccine	Coccidiosis infection	Canola	Phase II	Guardian Biosciences, Canada	Basaran and Rodríguez-Cerezo (2008)
Gastroenteritis virus (TGEV) capsid protein	Piglet gastroenteritis	Maize	Phase I	ProdiGene[b], USA	Basaran and Rodríguez-Cerezo (2008)
H5N1 vaccine candidate	H5N1 pandemic influenza	Tobacco	Phase I	Medicago, USA	http://www.medicago.com
Antibodies					
CaroRX	Dental caries	Tobacco	EU approved as medical advice	Planet Biotechnology, USA	http://www.planetbiotechnology.com/
DoxoRX	Side-effects of cancer therapy	Tobacco	Phase I completed	Planet Biotechnology, USA	http://www.planetbiotechnology.com/
RhinoRX	Common cold	Tobacco	Phase I completed	Planet Biotechnology, USA	http://www.planetbiotechnology.com/
Fv antibodies	Non-Hodgkin's lymphoma	Tobacco	Phase I	Large Scale Biology, USA	http://www.lsbc.com[a]
IgG (ICAM1)	Common cold	Tobacco	Phase I	Planet Biotechnology, USA	http://www.planetbiotechnology.com/
Antibody against hepatitis B	Vaccine purification	Tobacco	On market	CIGB, Cuba	Kaiser, 2008
Therapeutic human proteins					
Gastric lipase, Merispase®	Cystic fibrosis	Maize	On market	Meristem Therapeutics France	http://www.meristem-therapeutics.com
α-Galactosidase	Fabry disease	Tobacco	Phase I	Planet Biotechnology, USA	http://www.planetbiotechnology.com/
Lactoferon™ (α-interferon)	Hepatitis B and C	Duckweed	Phase II	Biolex, USA	http://www.biolex.com/
Fibrinolytic drug (thrombolytic drug)	Blood clot	Duckweed	Phase I	Biolex, USA	http://www.biolex.com/
Human glucocerebrosidase (prGCD)	Gaucher 's disease	Carrot suspension cells	Awaiting USDA's approval	Protalix Biotherapeutics, Israel	http://www.protalix.com/glucocerebrosidase.html
Insulin	Diabetes	Safflower	Phase III	SemBioSys, Canada	http://www.sembiosys.com/
Apolipoprotein	Cardiovascular	Safflower	Phase I	SemBioSys, Canada	http://www.sembiosys.com/
Nutraceuticals					
ISOkine™ , DERMOkine™	Human growth factor	Barley	On market	ORF Genetics	http://www.orfgenetics.com/
Human intrinsic factor, Coban	Vitamin B12 deficiency	*Arabidopsis*	On market	Cobento Biotech AS	http://www.cobento.dk/default.asp?id=76
Human lactoferrin	Anti-infection, anti-inflammatory	Rice	Advanced, on market as fine chemical	Ventria, USA	http://www.ventriabio.com/
Human lysozyme	Anti-infection, anti-inflammatory	Rice	Advanced, on market as fine chemical	Ventria, USA	http://www.ventria.com/
Immunosphere™	Food additive for shrimps	Safflower	Marketing expected for 2010	SemBioSys, Canada	http://www.sembiosys.com/

[a] LSBC filed bankruptcy in 2006.
[b] Prodigene has winded up activity.

Obembe et al, 2011