Veterinary vaccines produced in plants

Institute of Virology and Institute of Genetic CICVyA, INTA, Castelar, Argentina

Institute of Genetic and Molecular Biology INGEBI, Buenos Aires, Argentina

Workshop Argentina-Japan
"Bioscience and Biotechnology for the Promotion for Agriculture and Food Production"
August 3rd to 7th, 2009

PhD Andrés Wigdorovitz

Summary

Agriculture and Food Production in Argentina

Production of experimental vaccines in alfalfa transgenic plants

- Foot and mouse disease virus

  Development of transgenic alfalfa plants containing the foot and mouth disease virus structural polyprotein P1 and its utilization as an experimental immunogen.

- Bovine Rotavirus

  Passive protection against bovine rotavirus (BRV) induced by a peptide edible vaccine produced in transgenic alfalfa.

- Bovine diarrhea virus

  First report of an experimental vaccine against BVDV produced in alfalfa transgenic plants that induce neutralizing antibodies and protection in cattle.

Production of nanobodies (Llama –derived Single –Chain Antibody Fragments), in tobacco transplantomic plants.

ARGENTINA

- 3,760,000 km²
- Population~ 39 M
- Government:

  Representative Republican Federal
- Language

  Spanish

ARGENTINE PRODUCTION

NOA

- Sugar
- Tobacco
- Citrus
- Oilseeds: Soybean, Olive
- Wine
- Bovine Meat
- Vegetables
- Native Forest
- Honey
- Camelids
- Cotton

CUYO

- Pip Fruit
- Wine
- Oilseed: Olive
- Vegetables
- Bovine Meat

PATAGONIA

- Wool
- Ovine Meat
- Apple and Pear
- Berries
- Native Forest
- Bovine Meat
- Wine
- Honey

NEA

- Bovine Meat
- Cotton
- Oilseed: Soybean
- Implanted Forest
- Native Forest
- Yerba Mate (Ilex paraguaiensis)
- Tea
- Rice
- Citrus
- Vegetables
- Tobacco

PAMPEANA

- Wheat and Maize
- Oilseeds: Soybean-Sunflower
- Bovine Meat
- Bovine Milk
- Vegetables
- Porcine Meat
- Fruits
- Rice
- Poultry and Eggs
- Implanted Forest

AGRICULTURAL PRODUCTION

Grains:	**41,912 million tons**
Oilseeds:	**51,502 million tons**
Pip fruit:	**1,936 million tons**
Citrus:	**3,091 million tons**
Honey:	**115,000 tons**
Wines:	**15,400 million hectoliters**
Beef:	**3,117 million tons**
Pork:	**198,050 tons**
Poultry:	**1,160,000 tons**
Milk:	**9.600 million liters**
Wool:	**76.500 tons**

AGRICULTURAL EXPORTS

Agricultural and livestock exports: 23.870 million USD

- **First world exporter of flour and oil soybean, sunflower oil, honey, pears and lemon.**
- **Second world exporter of maize and sorghum.**

- **Third world exporter of soybean.**
- **Fifth world exporter of wheat and beef.**

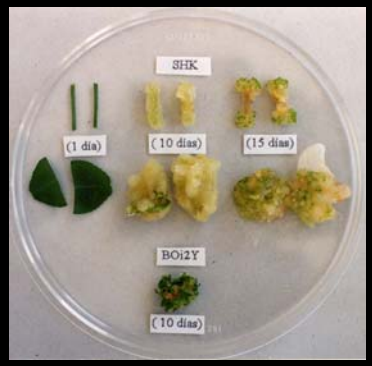
Gross Domestic Product: 213.172 million USD

Agricultural, Livestock and Agro-industry GDP: 30 %

INTA

- INTA Headquarters
- 15 Regional Centers
- 47 Experimental Stations
- 4 Research Centers
- 15 Research Institutes
- > 300 Extension Agencies
- 2 Private Organizations

Production of experimental vaccines in alfalfa transgenic plants



Alfalfa (*Medicago sativa*)

It is the most important forage grass in Argentine and it presents high levels of total soluble protein.

General plant advantages :

- Economic.
- Easy to obtain in big amounts.
- It can be used as an oral vaccine.

General plants disadvantages:

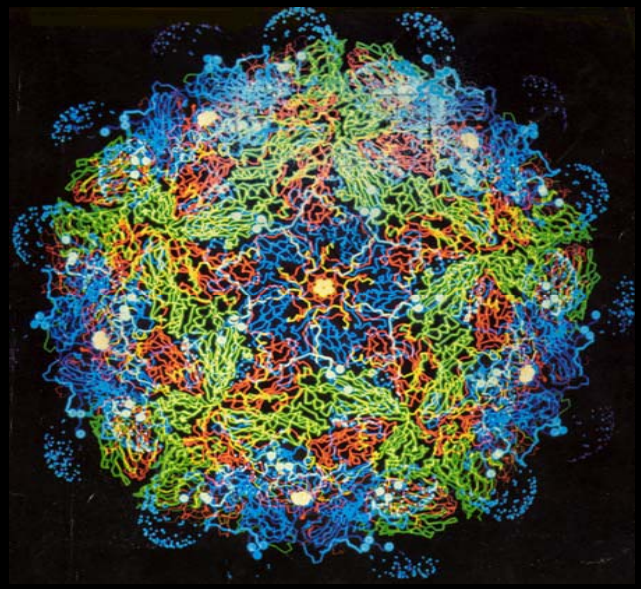
- Low levels of heterologue protein expression.

INTA

Introduction

FOOT AND MOUTH DISEASE VIRUS

It is responsable for the most important economic losses of cattle production in the world



FMDV is a picornavirus with a single-stranded positive sense RNA genome.

Icosaedrical capsid, 30nm size

4 structural proteins compose its capsid:
VP1, VP2, VP3 and VP4.

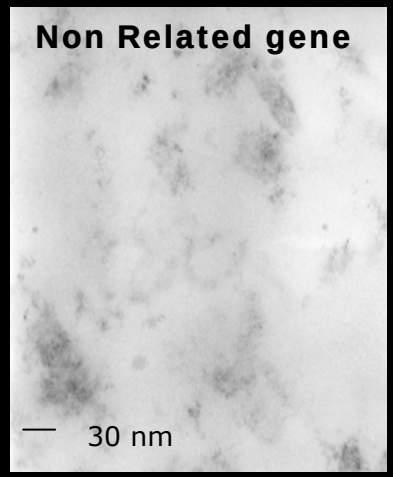
INTA

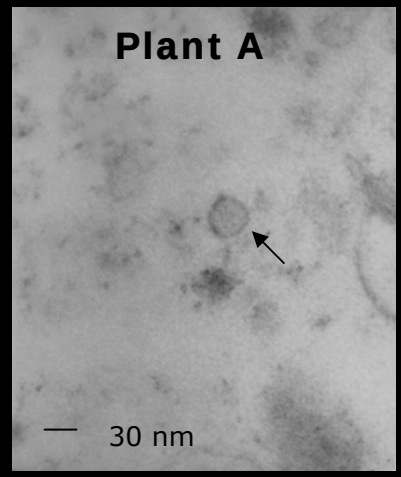
Objectives

- Expression of P1 polyprotein of Foot and Mouth Disease virus in transgenic alfalfa plants for the production of experimental vaccines.
- Evaluation in a murine model.

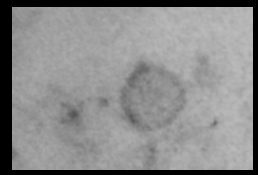
Construction	# transformed plants
pROK-P1.2A.2B.3C	20

Electronic microscopy





Plant A

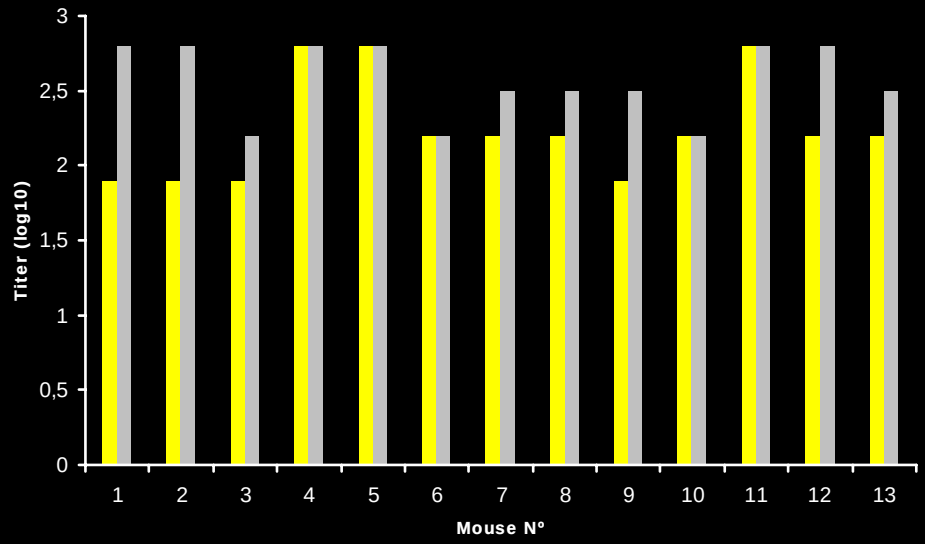


FMDV empty capsid

Antibody response

ELISA

Immune response in mice I.P. immunized with P1 polyprotein transgenic alfalfa plants



0,05- 0,2 mg/g TSP

Neutralizing assay

Detection of neutralizing antibodies induced in I.P immunized mice with plant A extracts

Mice IP immunized with:	Mouse Nº*	SNI
Alfalfa Plant A	1	2.2
	2	2.3
	3	2.1
	4	2.1
Alfalfa expressing a non related gene	5	0.6
	6	0.6

Murine model

Protection against FMDV challenge in mice immunized with P1 polyprotein transgenic plants

Mice IP immunized with:	Protection (%)	
Alfalfa Plant A	13/13 (100%)	9/9 (100%)
Alfalfa expressing a non related gene	0/10 (0%)	0/6 (0%)
Buffer	0/6 (0%)	0/6 (0%)

Immunology and Cell Biology (2005) 83, 1–000 doi:10.1111/j.1440-1711.2005.01338.x

Special Feature

Transgenic plants for the production of veterinary vaccines

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ELSEVIER

Vaccine (2004)

Vaccine

www.elsevier.com/locate/vaccine

Development of transgenic alfalfa plants containing the foot and mouth disease virus structural polyprotein gene P1 and its utilization as an experimental immunogen

María J. Dus Santos[a], Consuelo Carrillo[c], Fernando Ardila[b], Raúl D. Ríos[b], Pascual Franzone[b], María E. Piccone[c], Andrés Wigdorovitz[a,*], Manuel V. Borca[c,**]

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pending patent P040102842

Introduction

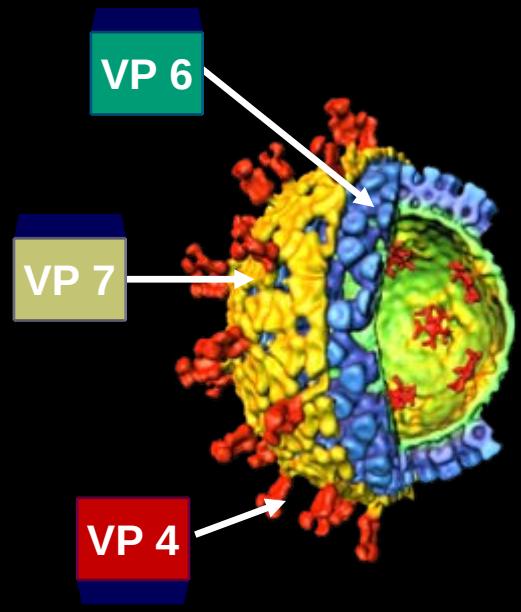
BOVINE ROTAVIRUS

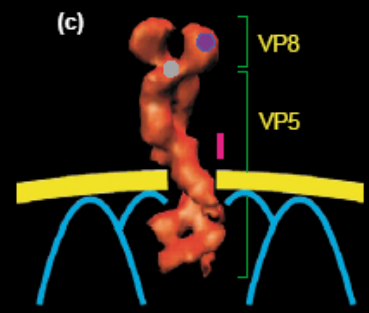
Rotavirus infections are the primary cause of severe diarrhea in young animals and children in the world.

BRV is a rotavirus with a RNA positive genome.

2 structural proteins compose its outer capsid: VP4, and VP7.

VP4 and VP7 carry critical epitopes responsible for the induction of neutralizing antibodies.

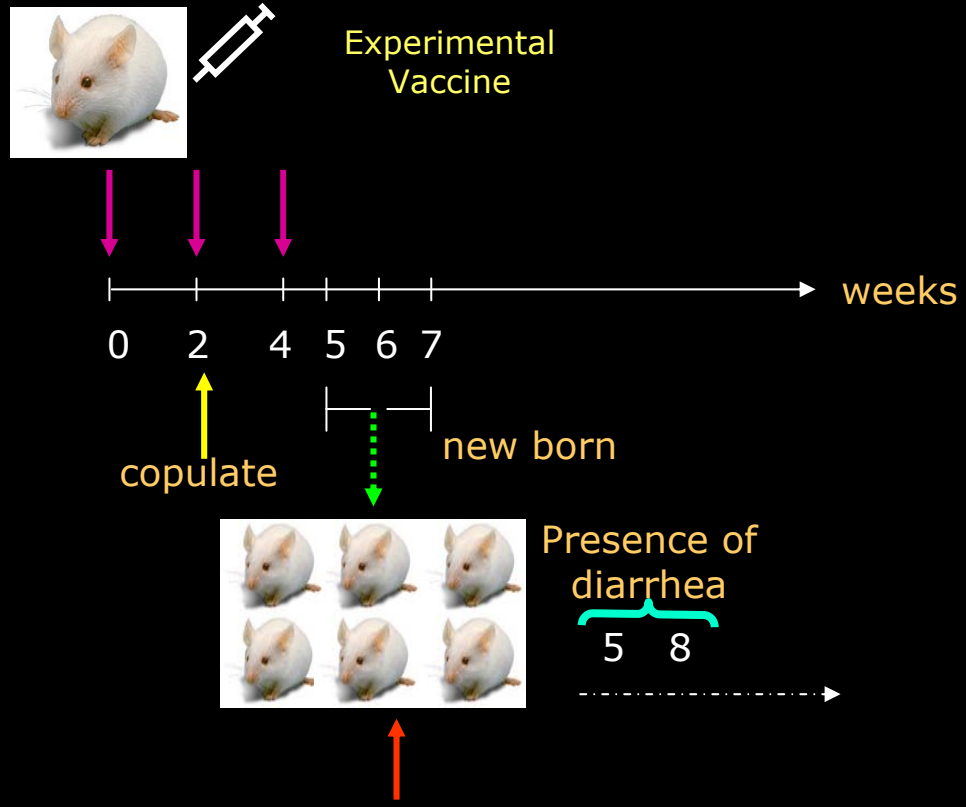




Objective

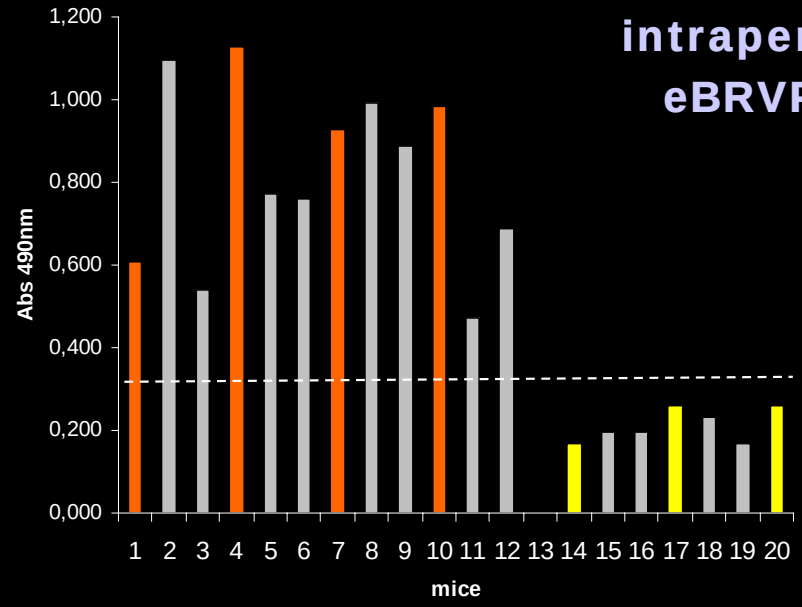
- To develop an experimental edible vaccine based on the use of recombinant BRV peptide fused to the enzyme β-GUS expressed in transgenic alfalfa plants.
- To assess the ability of the chimerical protein to induce passive protective immunity against virulent bovine rotavirus in suckling mice.

Murine model



Antibody response

Immune response in mice intraperitoneally immunized with eBRVP4-BGUS leaves extracts



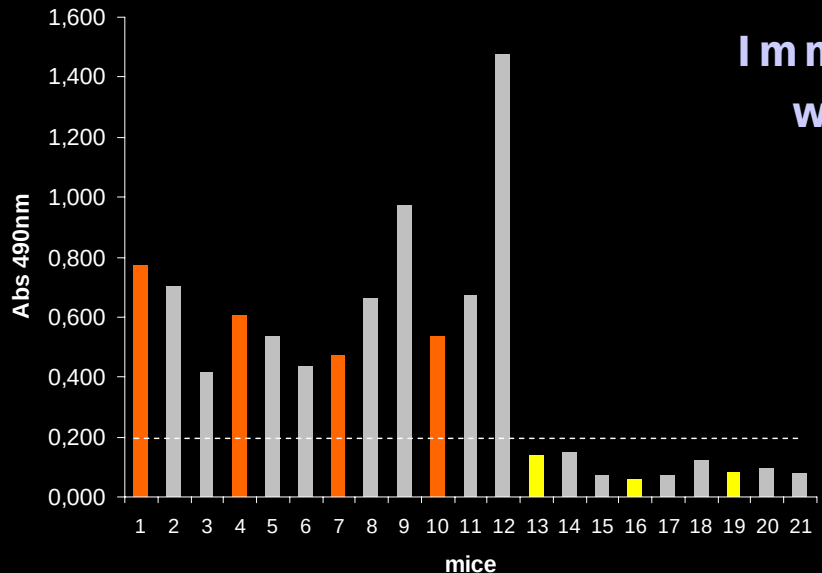
vaccinated dams

respective offspring

Negative control dams

ELISA

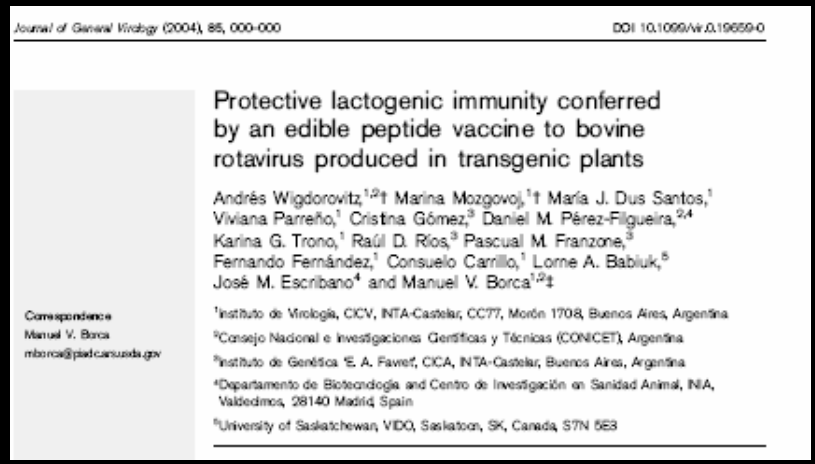
Immune response in mice fed with eBRVP4-BGUS fresh leaves



Murine model

Evaluation of passive protection against BRV C486

Mice IP immunized with:	Route of immunization	Protection (%)	Fisher´s Test
Alfalfa expressing *eBRV4-β* GUS	Oral	22/29 (76%)	p=0.003
	IP	10/14 (71%)	P= 0.0063
Alfalfa expressing a non related gene	oral	4/19 (21%)	p=0.003
	IP	2/13 (21%)	P= 0.0063



Journal of General Virology (2004), 85, 000-000 DOI 10.1099/vir.0.19659-0

Protective lactogenic immunity conferred by an edible peptide vaccine to bovine rotavirus produced in transgenic plants

Andrés Wigdorovitz,[1,2]† Marina Mozgovoj,[1]† María J. Dus Santos,[1] Viviana Parreño,[1] Cristina Gómez,[3] Daniel M. Pérez-Filgueira,[2,4] Karina G. Trono,[1] Raúl D. Ríos,[3] Pascual M. Franzone,[3] Fernando Fernández,[1] Consuelo Carrillo,[1] Lorne A. Babiuk,[5] José M. Escribano[4] and Manuel V. Borca[1,2]‡

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[5]University of Saskatchewan, VIDO, Saskatoon, SK, Canada, S7N 5E3

Introduction I

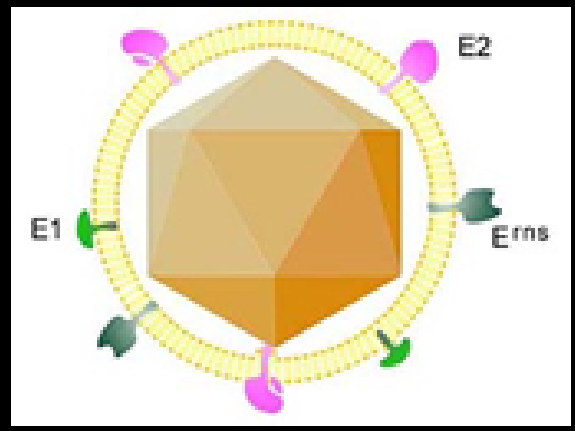
BOVINE DIARREA VIRUS

Infects bovines of all ages, causing reproduction problems and altering biological products of high commercial value thereby, resulting in considerable economical losses.

BVDV is a pestivirus with a RNA positive genome.

Spherical shape 40 -60 nanometers (nm) of diameter, icosaedral capsid and external envelope.

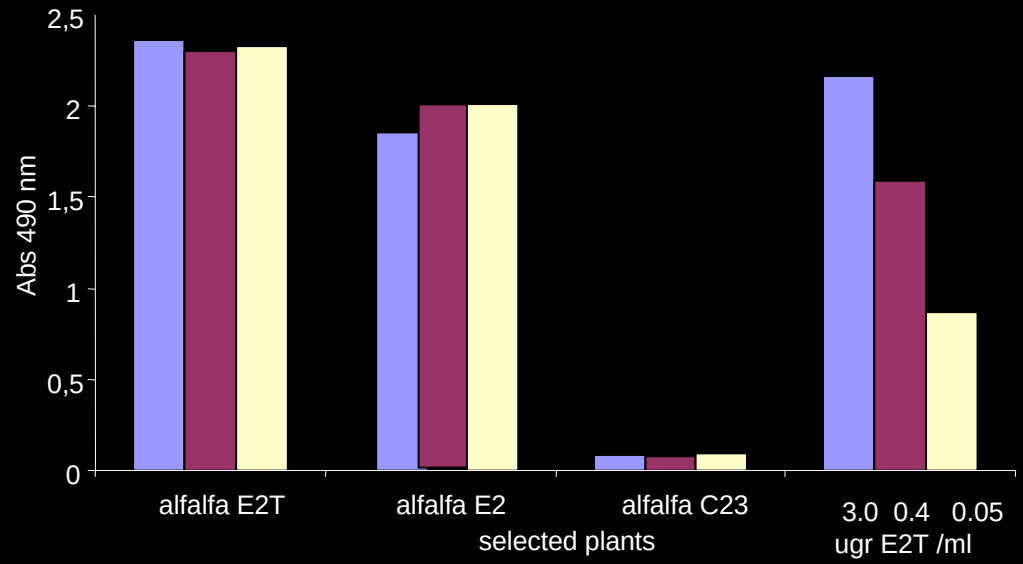
E2 glycoprotein carries critical epitopes responsible for the induction of neutralizing antibodies.



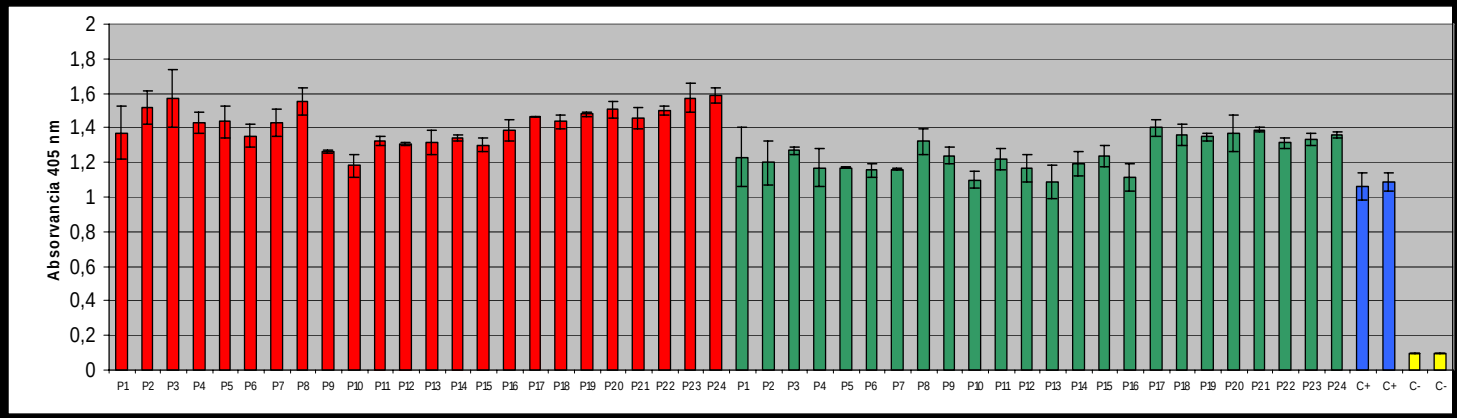
INTA

Plants expression I

Quantification and stability of the selected Transgenic plants obtained using *Agrobacterium tumefaciens* - mediated transformation of alfalfa

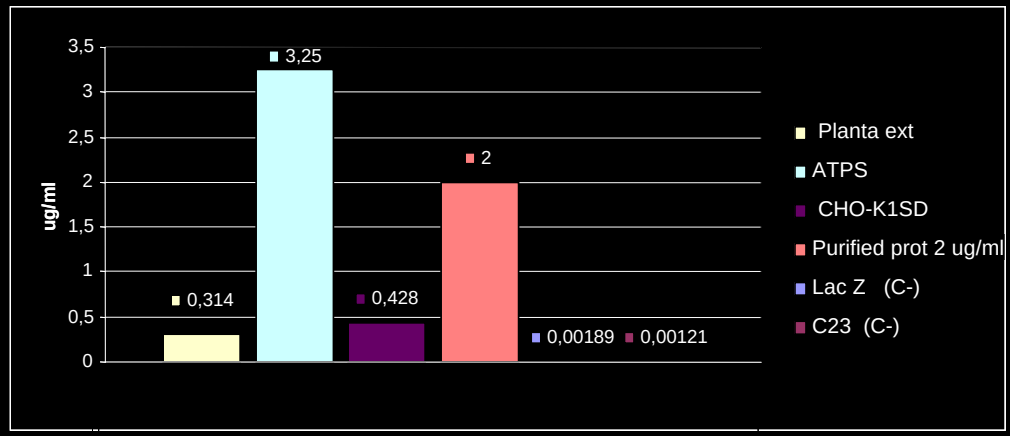


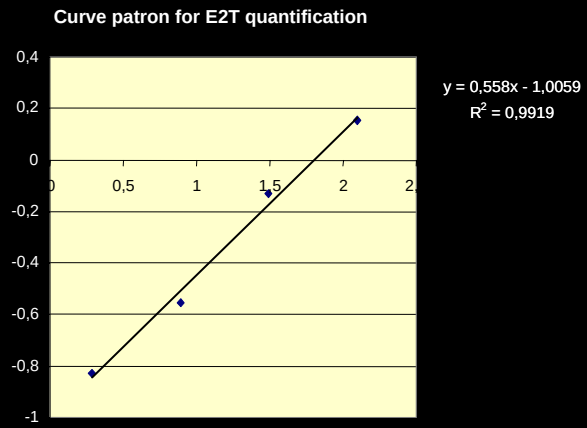
E2T: 1.7 ugr /gr leaf
E2: 1,5 ugr/ gr leaf

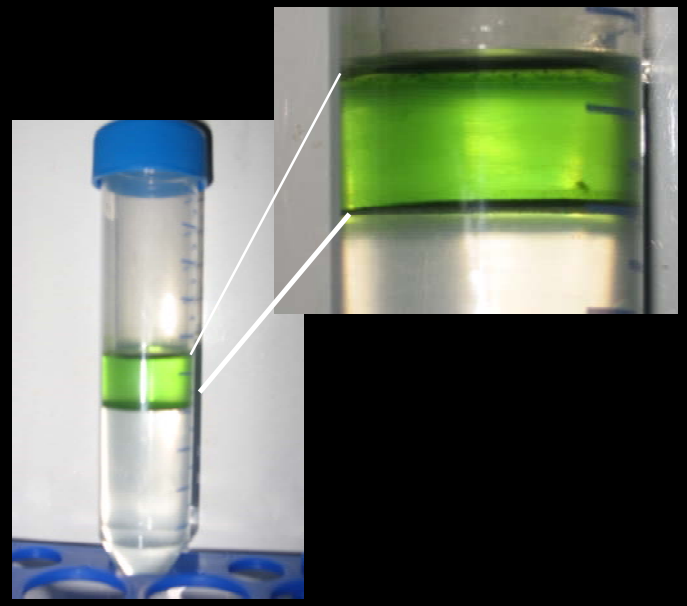


Plants expression I

Aqueous two-phase systems for purification of recombinant E2T and APCH1-E2T proteins expressed in alfalfa plants



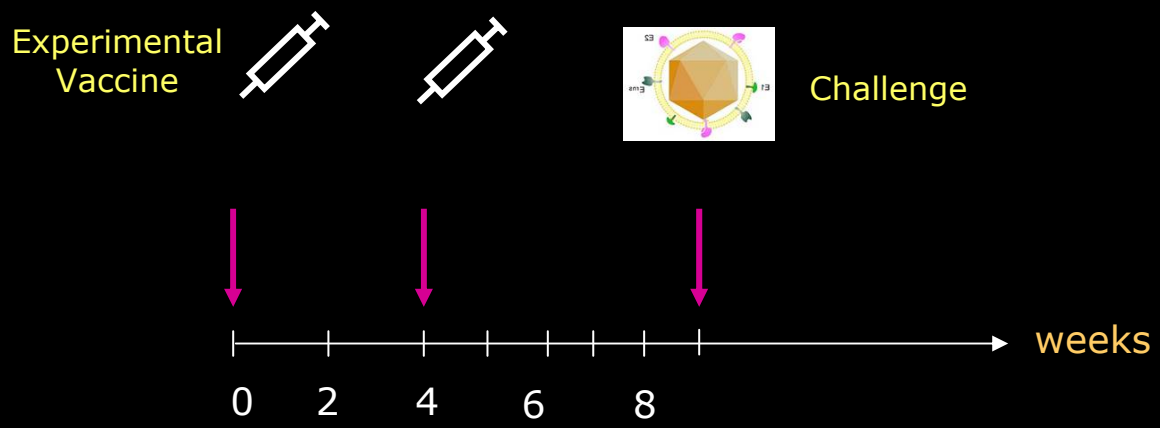




Antibody Response I

Evaluation of the immunogenicity of the E2T protein expressed in alfalfa plants in cattle

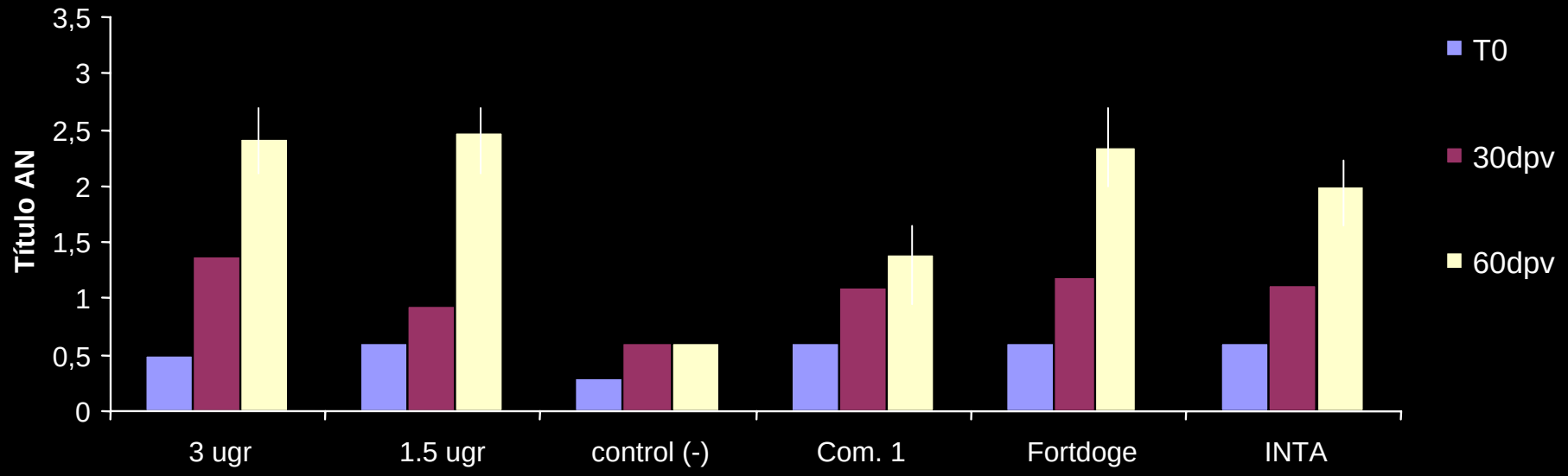
GROUP	ANTIGEN	n	CATTLE DOSIS
1	E2T cc alfalfa	3	3 ug
2	E2T cc alfalfa	3	1,5 ugr
3	BVDV INTA 10∂/ml	3	-
4	Negative control (C23-VP8)	3	3 ug



Antibody Response I

Evaluation of the immunogenicity of the E2T protein expressed in alfalfa plants in cattle

Viral neutralization test



Challenge I

Evaluation of the protection induced by the experimental vaccines in cattle

Six-month–old Hereford calves were challenge two weeks after the last immunization with 25 ml 1 X 10 -6 TCID 50/ml BVDV 98/124 type 1 by aerosolization

We have the approbation from CONABIA and SENASA for the immunization and challenge experience.

Challenge I

Evaluation of the protection induced by the experimental vaccines in cattle

Viirus shedding after BVDV challenge					
Antigen					
	DPI 0	**DPI 4**	**DPI 6**	**DPI 8**	**DPI 11**
mammalian E2t 1,5 ug	0/3	2/3*	1/3	1/3	0/3
Alfalfa E2t 3 ug	0/3	0/3	0/3	0/3	0/3
Alfalfa E2t 1.5 ug	0/3	0/3	1/3	1/3	0/3
BVDV Inact. ($10^{6.5}$)	0/3	0/3	0/3	0/3	1/3
Ctrl	0/3	3/3	2/3	1/3	0/3
* Virus isolation from either plasma or buffy coat. DPI: Days Post-Infection.					

INTA

pending patent P 090100556

Objective II

- **To express a fusion protein between a Single Chain Antibody Fragment (APCH1) against MHC class II using Cassava promoter, in order to optimize the accessibility of the recombinant protein to the immune system**

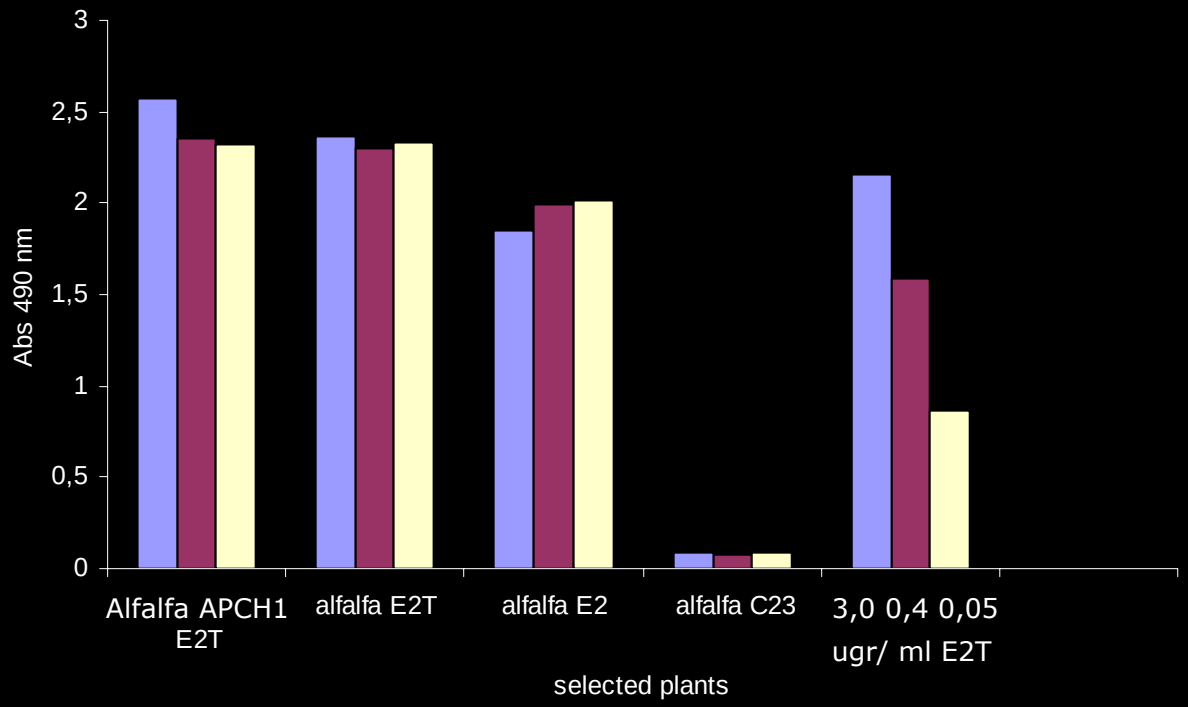
PCT/ES2008/070053.
Pending patent P080101073

Agreement ALGENEX – INTA.

Plants expression II

Transgenic plants obtained using *Agrobacterium tumefaciens* - mediated transformation of alfalfa

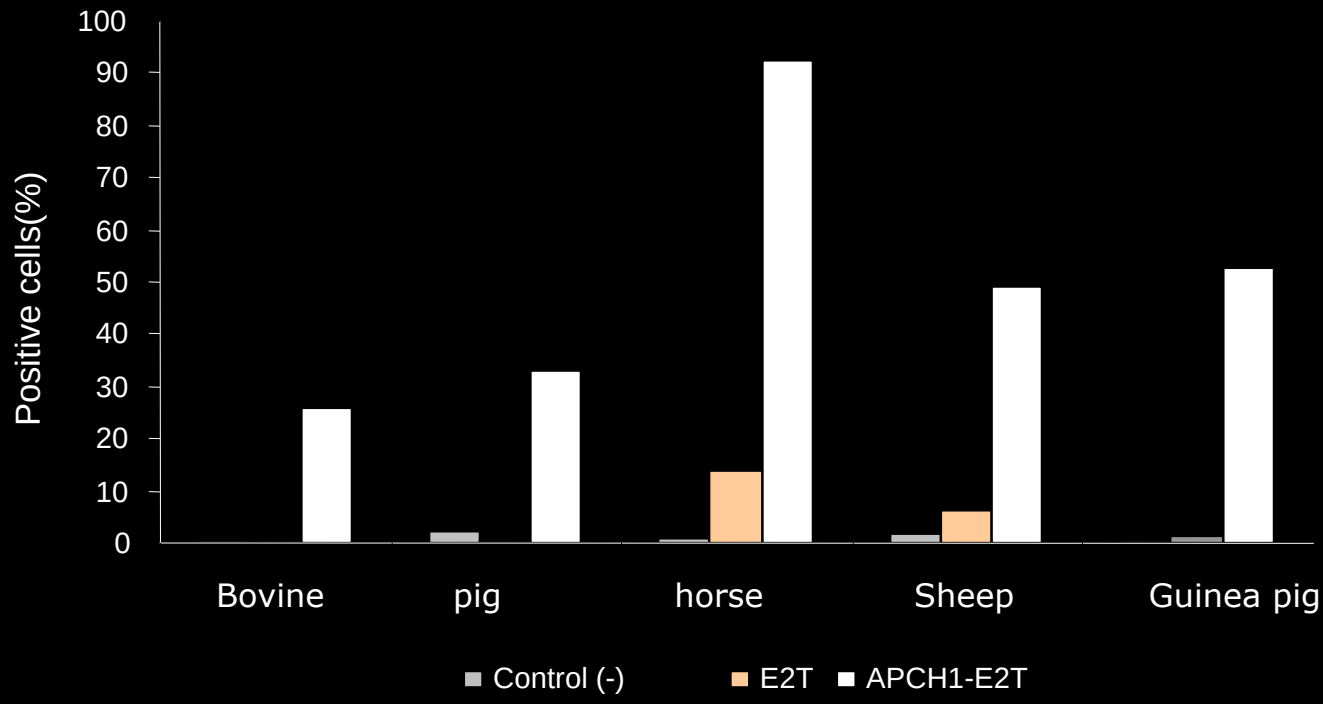
Construction	# transformed plants
pCar (cassava) –E2	33
pCar (cassava) –E2t	9
pCar (cassava) APCH1–E2t	12

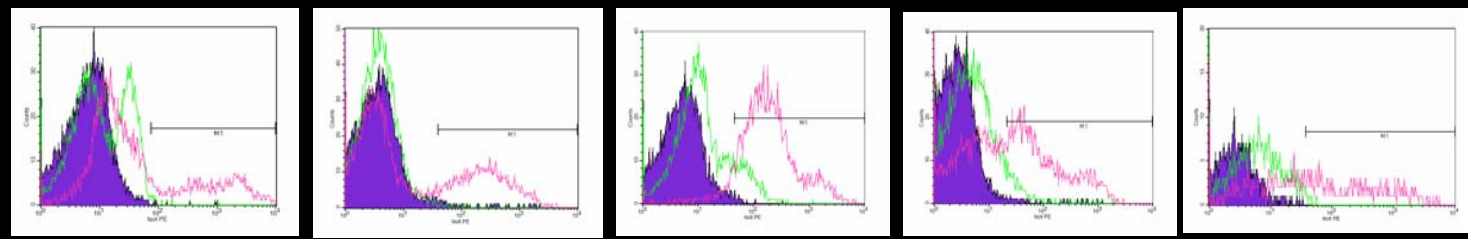


APCH1 E2T and E2T: 1.7 ugr /gr leaf
E2: 1,5 ugr/ gr leaf

Flow cytometry

Binding of APCH1-E2T to MHCII surface of peripheral blood mononuclear cells (PBMCs) from different species by flow cytometry





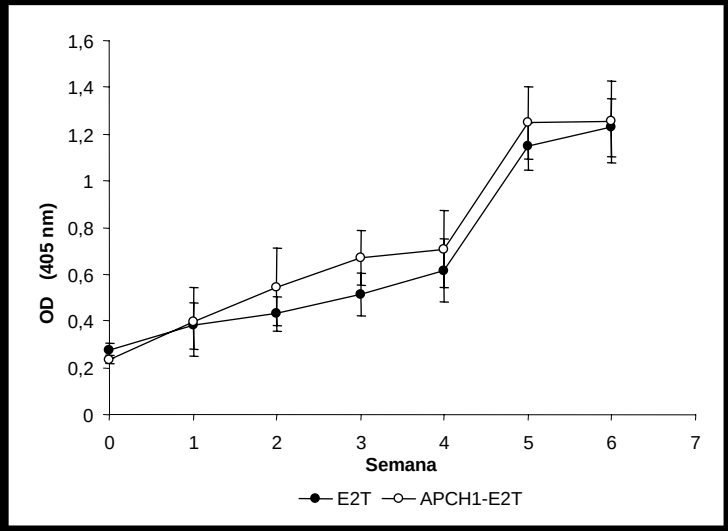
Antibody Response II

Comparison of the immunogenicity of the E2T and APCH1-E2T protein expressed in alfalfa plants in cattle

GROUP	ANTIGEN	n	CATTLE DOSIS
1	E2T	3	1 ug
2	APCH1- E2T	3	1 ug
3	E2T	3	0,2 ug
4	APCH1- E2T	3	0,2 ug
5	Negative control	3	1 ug

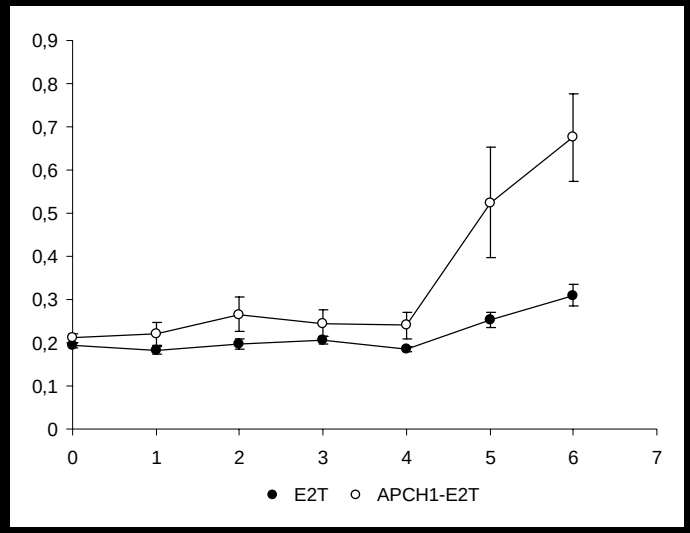
ELISA

Dosis 1 ug



Booster Week 4 (dilution 1/40)

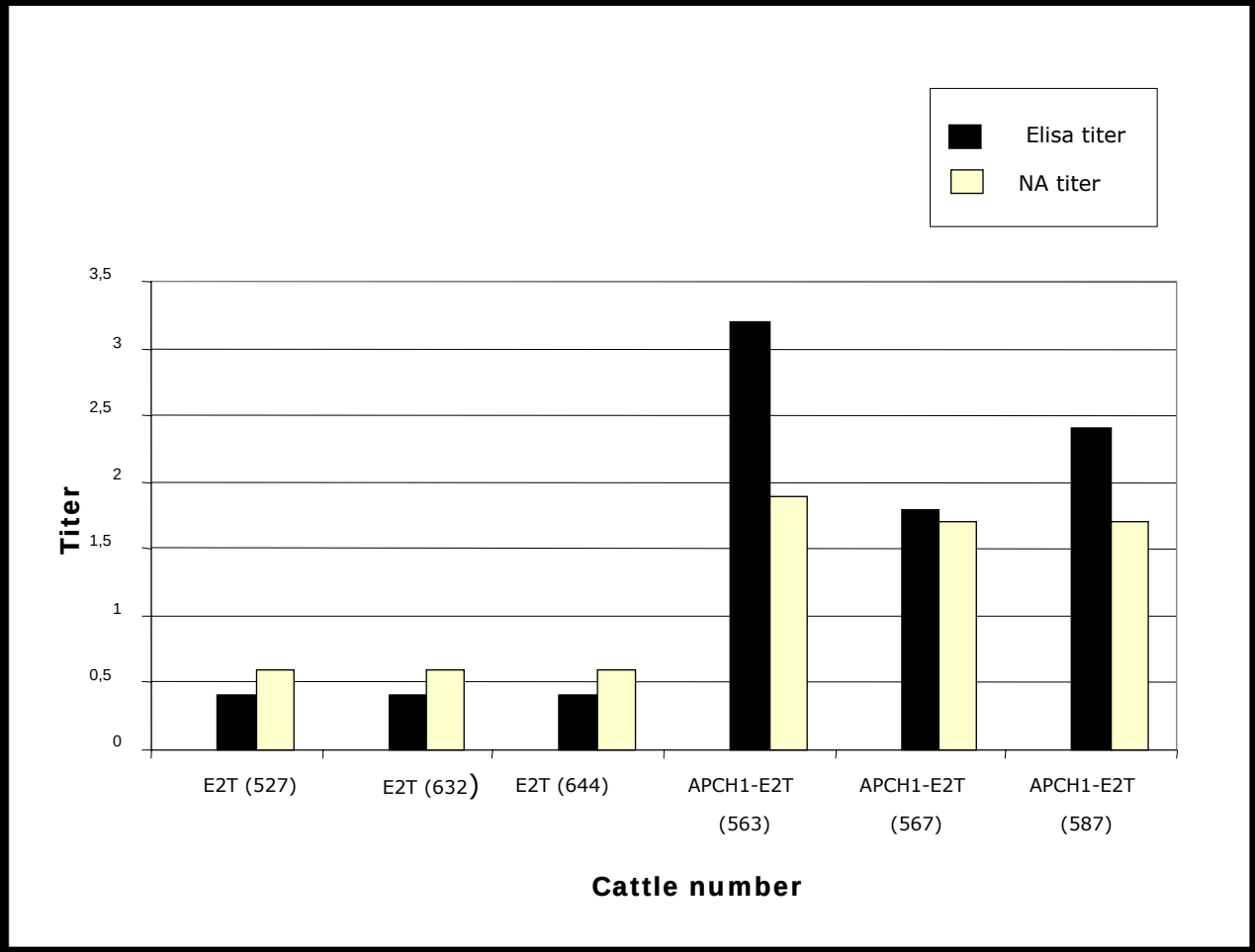
Dosis 0.2 ug



Booster week 4 (dilución 1/40)

Antibody Response II

Comparison of the immunogenicity of 0,2 ug of E2T and APCH1 E2T protein expressed in alfalfa plants in cattle

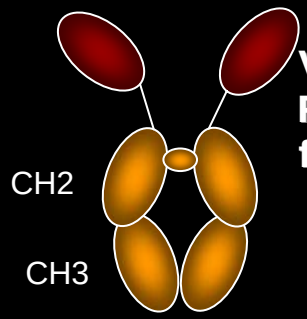


Conclusions

- It was possible to express E2T and APCH1-E2T protein in alfalfa transgenic plants
- Without any concentration steps we obtain 1,5 mgr/ kgr of E2t in alfalfa plants
- Results obtained allow us to establish the conditions for recuperation of both protein E2T and APCH1-E2T with high yield, low cost and the possibility to carry out the scale-up easily.
- E2t experimental vaccine induce neutralizing antibodies and protection in cattle
- APCH1-E2T recognize the MHCII molecule on the surface of peripheral blood mononuclear cells (PBMCs) from different species
- APCH1 E2T optimize the immune response in cattle

INTA

Production of nanobodies, in tobacco transplantomic plants.



VHH:
Fragment corresponding to a variable portion of light chain from camelidae

- Small sice molecule (15KDa)
- simple structure
- High thermic and chemic resistance
- Recombinant monoclonal antibodies

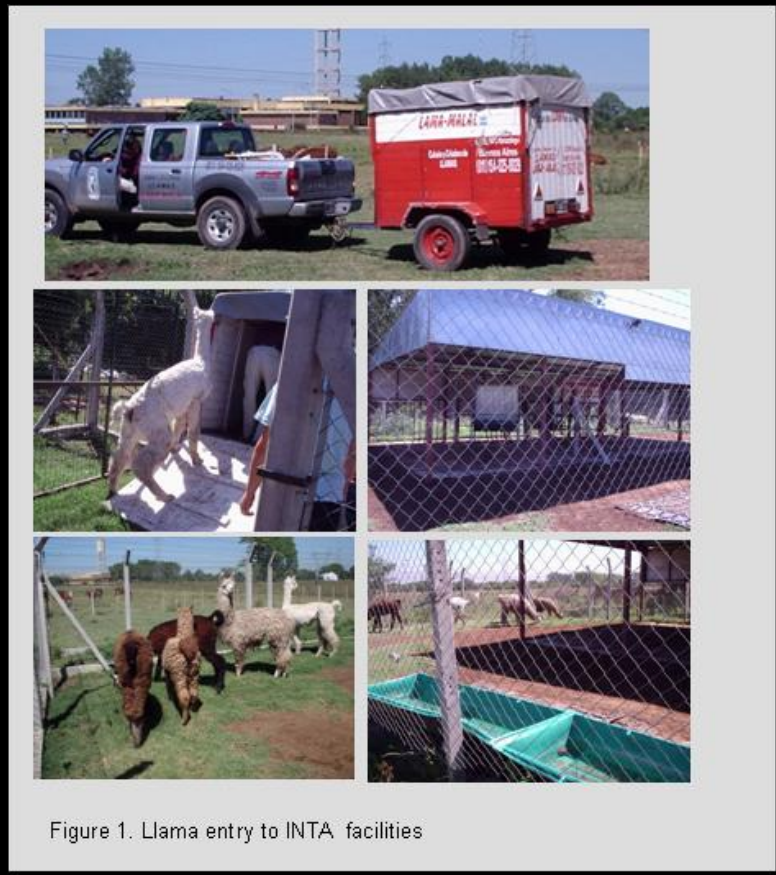


Figure 1. Llama entry to INTA facilities

INCUINTA

VHH TECNOLOGY

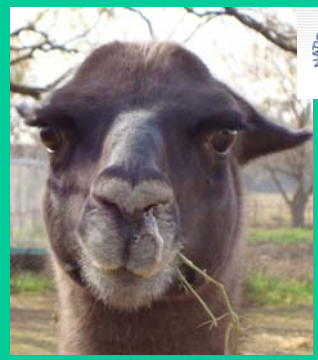
INTA

INSTITUTO LELOIR
FUNDACIÓN

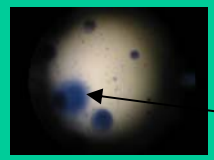
Inmunova

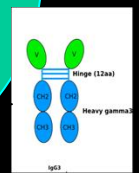
Ablynx

Wiener lab
GROUP
Investigación y tecnología al servicio de la salud



National Institutes of Health
The Nation's Medical Research Agency





MONOMERIC VHH ANTIBODIES AGAINST ROTAVIRUS VP6 FOR VIRUS DETECTION AND PASSIVE PROTECTION

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(54) Title: MONOMERIC VHH DOMAIN DERIVED FROM ANTI-VP6 CAMELID ANTIBODIES, DIMERIC DOMAIN, IMMUNISATION METHOD, ROTAVIRUS DETECTION METHOD, COMPOSITION, PREVENTION AND TREATMENT METHODS FOR ROTAVIRUS INFECTIONS

(57) Abstract: Monomeric VHH domain derived from anti-VP6 camelid antibodies, dimeric domain, immunisation method, rotavirus detection method, vaccine composition, prevention and treatment methods for rotavirus infections, wherein said domain may be any of the amino acid sequences shown in SEQ ID No. 1, SEQ ID No. 2, SEQ ID No. 3 or SEQ ID No. 4, and wherein said domains bind to protein VP6 of Group A rotavirus.

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INCUINTA

VHH TECNOLOGY

ALGENEX
Alternative Gene Expression

INTA

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0022-538X/08/$08.00+0 doi:10.1128/JVI.00436-08

Llama-Derived Single-Chain Antibody Fragments Directed to Rotavirus VP6 Protein Possess Broad Neutralizing Activity In Vitro and Confer Protection against Diarrhea in Mice▽

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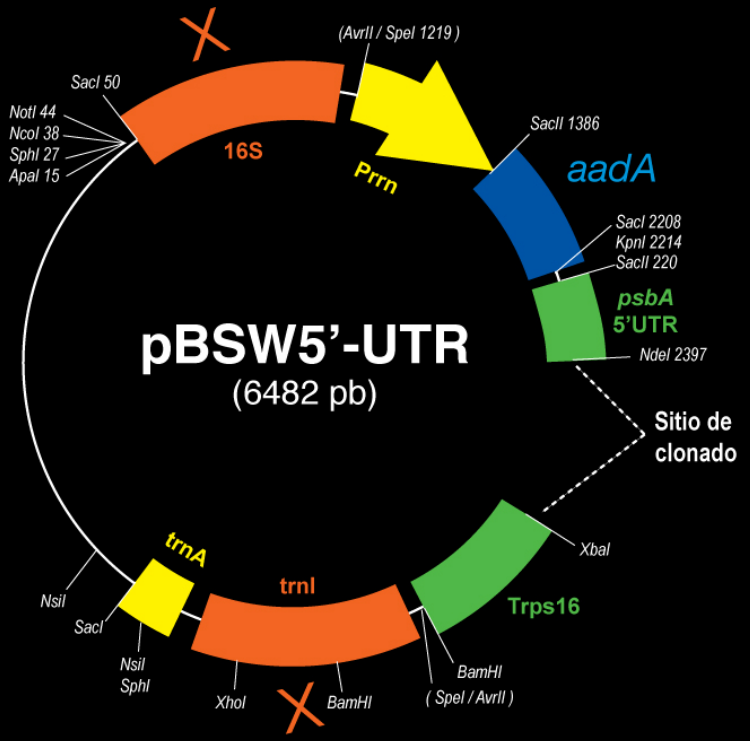
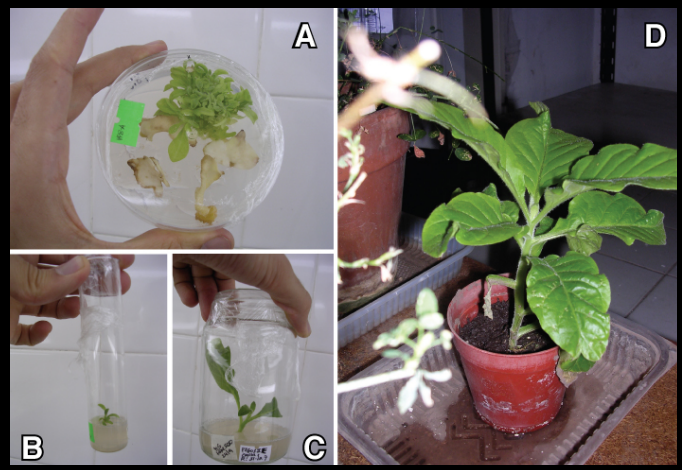
Chloroplast transformation





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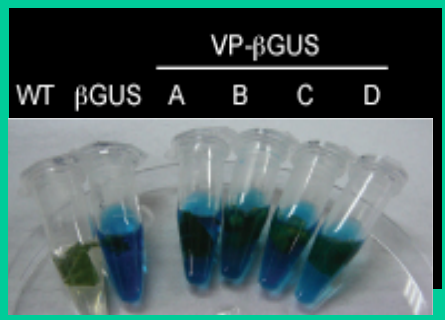
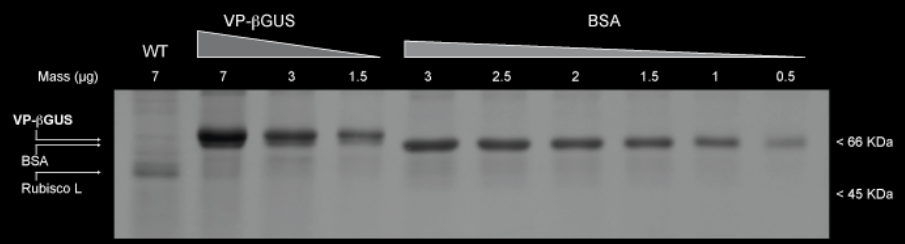
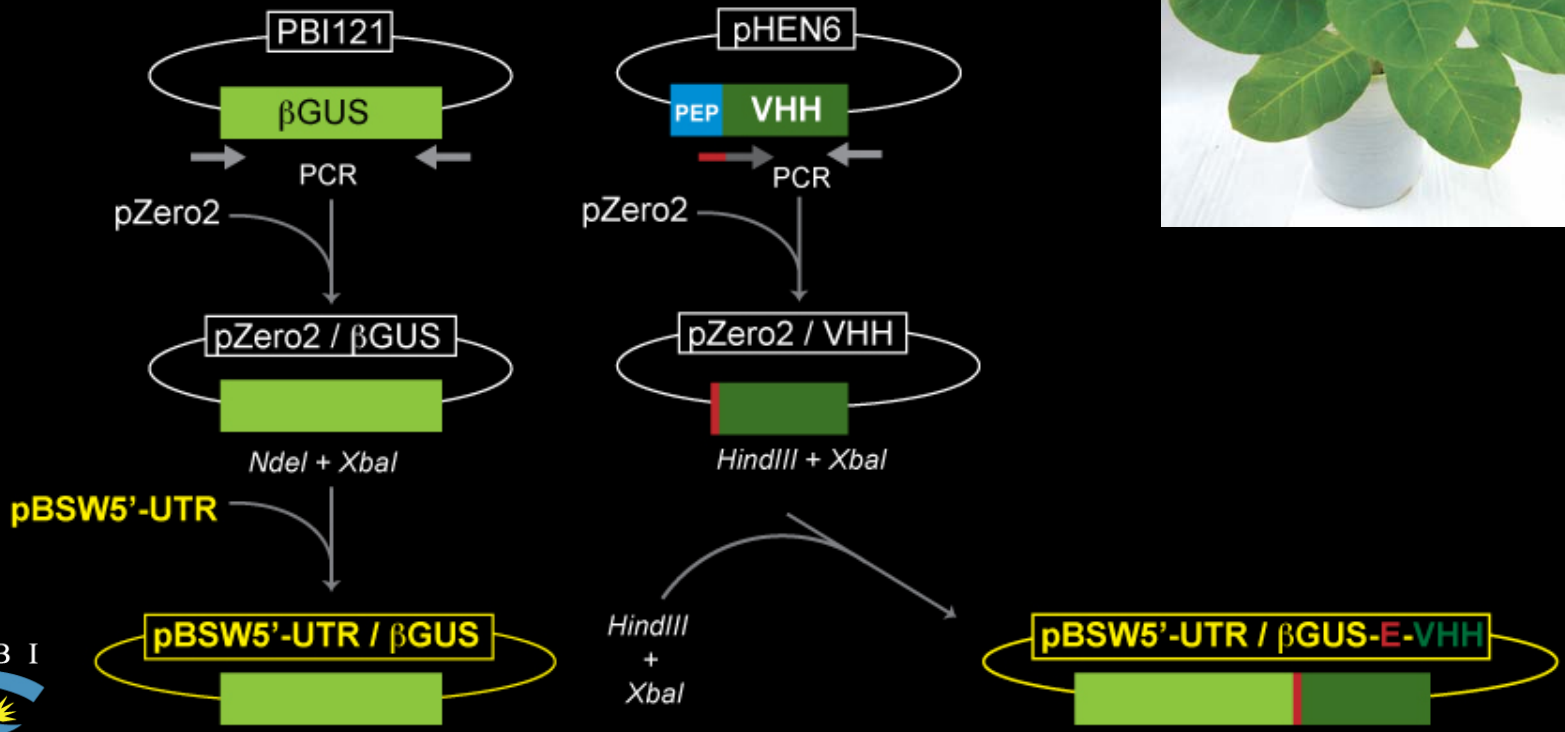




Construction pBSW5'UTR / β GUS-E-VHH



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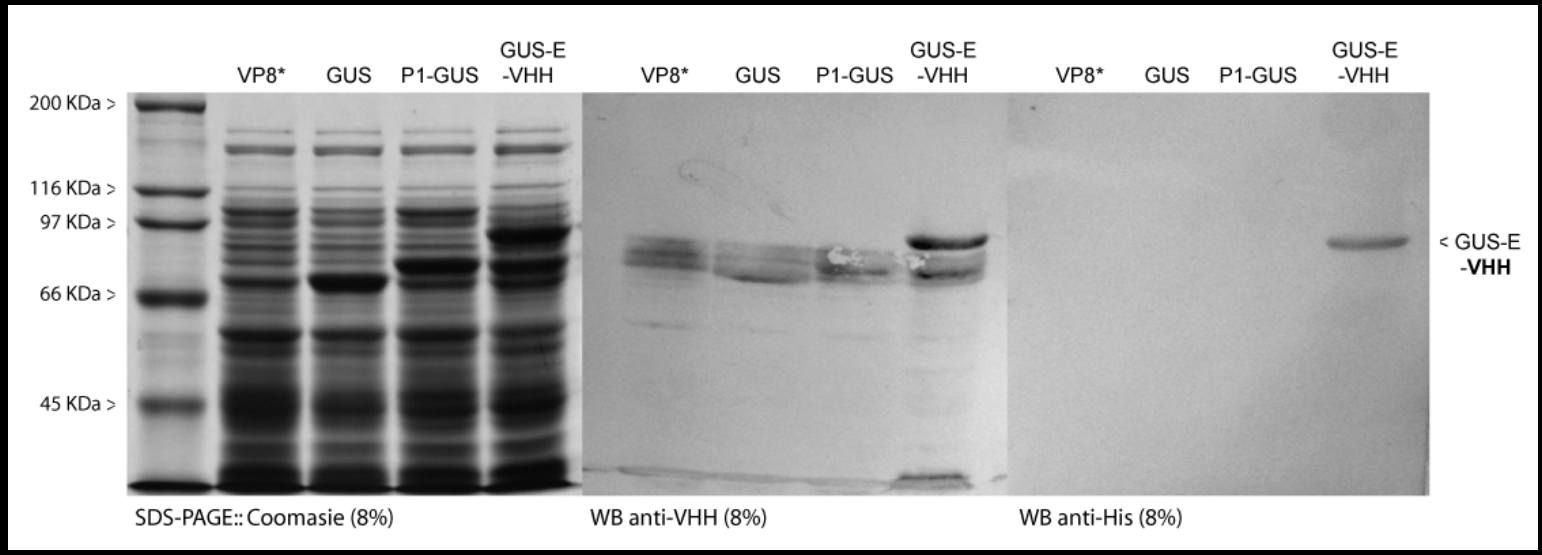

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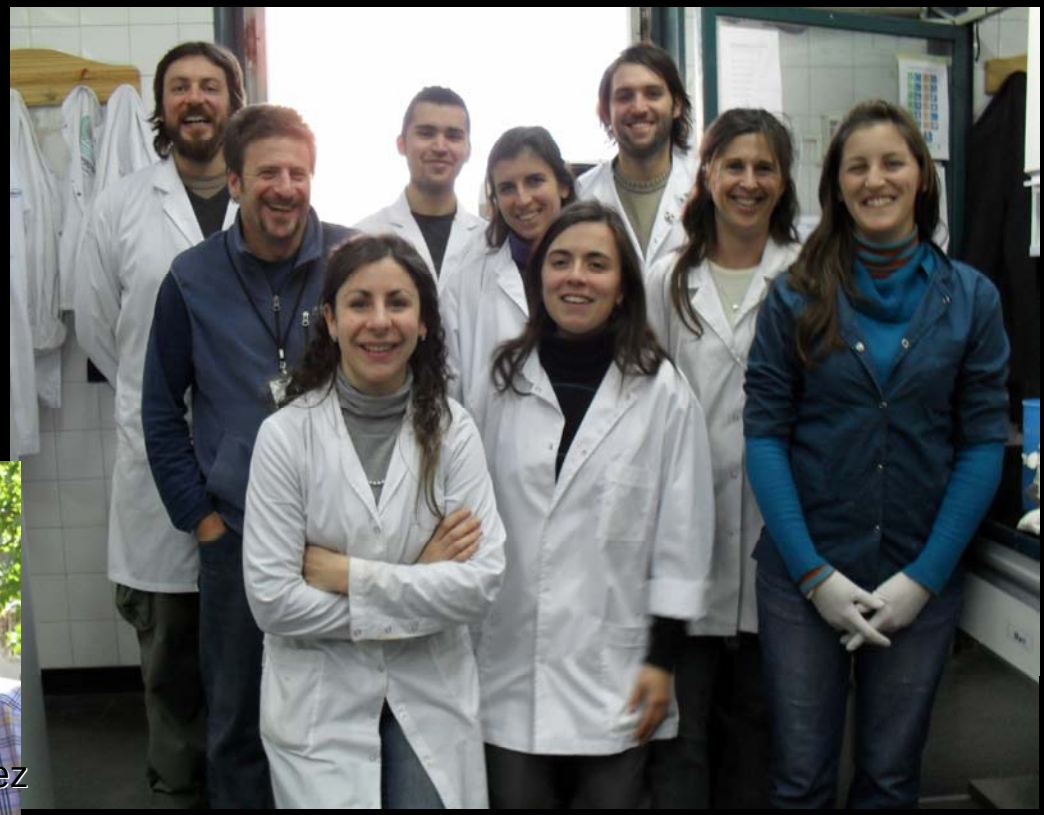
Production of antigens and antibodies for veterinary applications in tobacco transplastomic plants.

Viviana Parreño

Fernando Fernandez



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