Development of a plant-based vaccine in maize against Mokola virus

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Introduction

Rabies Zoonotic disease which causes 55.000 annual deaths in humans worldwide.

Family: *Rhabdoviridae*

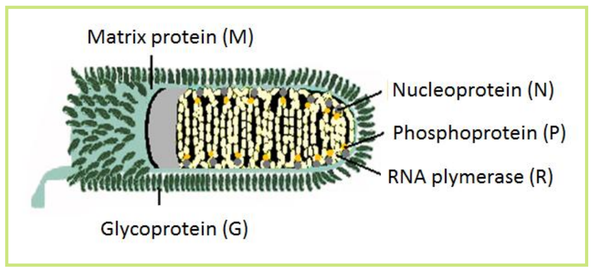
Genus: *Lyssavirus* (genotype 3)

Mokola virus (MOKV)

- Exclusively found in Africa.
- Never isolated in bats.
- Reported cases since 1968.
- Genetically the most distant from rabies virus.

Rabies virus vaccination fail to protect against MOKV.

MOKV structural organization:



Plant-based vaccines

Advantages
Cost efficiency.
No need for “cold chain”.
Eliminate cost of syringes and needles.
Reduced need of medical assistance in administration.
Oral application.
Ideal for veterinary use.
Mucosal and serum immune response.
Reduced concerns due to the non use of pathogens

Disadvantages
Low levels of protein expression (~1% TSP).
Need of high levels of proteins in animals.
Protein degradation in intestinal tract.
Need for protein purification.

Previous plant–based vaccine for animal rabies:

Production system	Maximum accumulation level
Tomato leaves and fruit	0.001% TSP
Tobacco leaves	0.38% TSP
Cantaloupe melon fruits	1.2% TSP
Carrots	1.2% TSP
Maize	1% TSP

Objectives

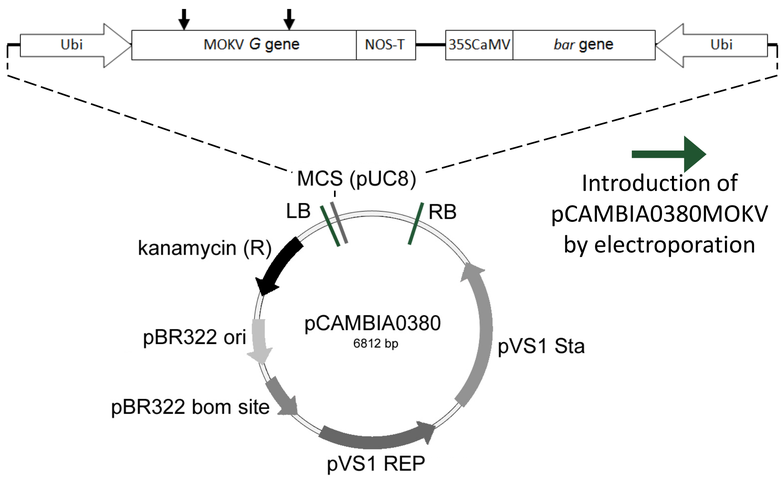
General objective

Development of a cheap and efficient vaccine using a plant expression system against MOKV by oral immunization.

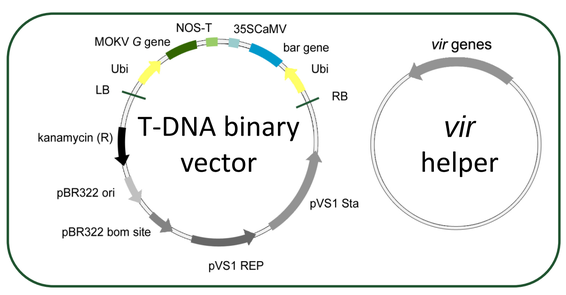
Specific objectives

- To obtain a transgenic plant which contains the G glycoprotein of MOKV.
- To observe normal growth and phenotype in the genetically modified plant.
- To find the optimal dosage for the immunization in mice.
- To maximize the accumulation of G protein >1% of TSP.
- To achieve a significant difference in the survival between mice immunized with the plant based-vaccine and mice immunized with Rabipur.

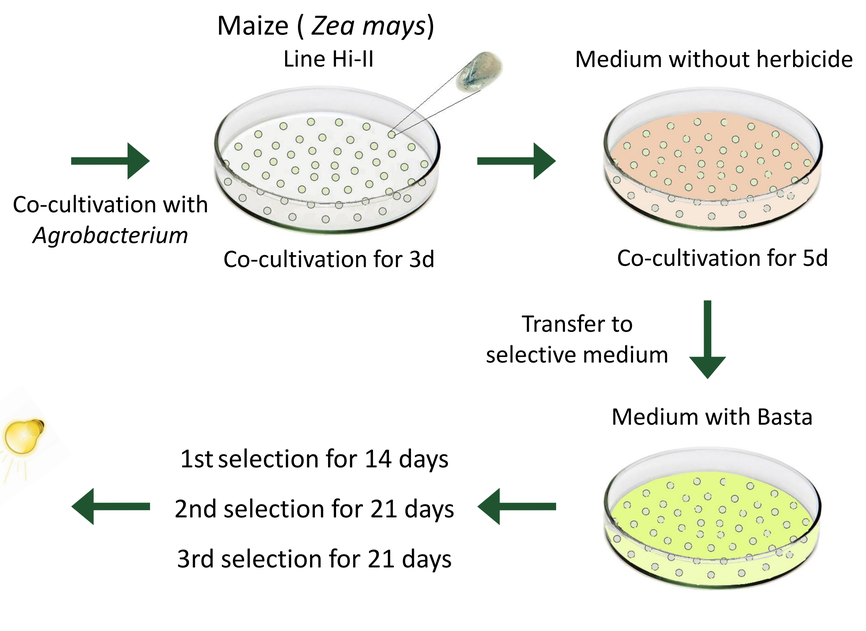
Material and methods



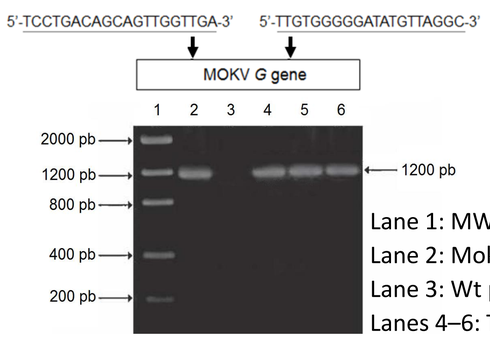
Transformation by binary vector systems



Agrobacterium tumefaciens. Strain LBA4404

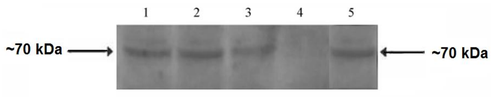


PCR analysis



Lane 1: MW.
Lane 2: Mokola virus.
Lane 3: Wt plant.
Lanes 4–6: Transformed plants.

Western blot analysis



Lanes 1–3: Transformed plants.
Lanes 4 / 5: negative / positive control.

Quantification on G protein by densitometry

Immunization o Balb/c mice

1st group: 1g of G protein / mice.
2nd group: 3g of G protein / mice.
3rd group: Rabipur.
Control group: wt maize kernels

Statistical analysis

Expected results

- pCAMBIA380MOKV would be obtained successfully.
- The *G* gene would be detected by PCR in the transformed plants.
- The G protein would be identified and quantified.
- To observe a clear difference in health and behavior between the mice immunized with transformed maize and mice immunized with Rabipur.
- The plant expression system would present significant difference ($p < 0.05$) in survival compared with Rabipur.
- Both mice groups orally immunized with 1g and 3g of G protein would be protected against MOKV.

Bibliography

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