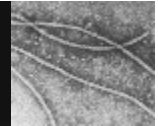


Evolutionary pathways of *Potato virus Y* adaption to the *va* resistance gene in *Nicotiana tabacum* and impacts for resistance management



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Biology and Evolution of RNA plant viruses



■ Economic losses due to pathogens in tobacco:

∅ Many pathogens = viruses, fungi, nematodes, pests

∅ Main viral pathogen on tobacco: *Potato virus Y* (PVY)



Mosaic

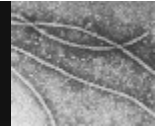


Vein necrosis

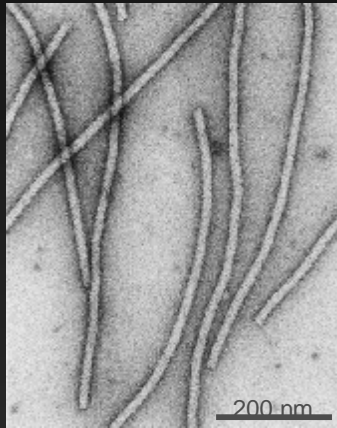


**Unconventional
symptom**

Yield losses: up to 100%



■ *Potato virus Y (PVY) : Potyviridae* family

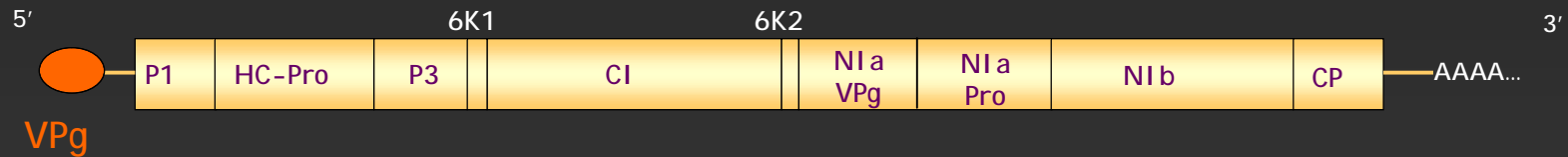


Ø Filamentous and flexuous particle (700-900 nm)

Ø Structure:

- ❖ Single-stranded RNA of 10kb
- ❖ Coat protein (CP)
- ❖ VPg protein linked in 5'

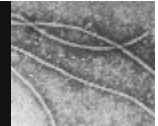
Ø Poly-protein cleaved in 10 proteins



Ø Transmitted in a non persistent manner by aphids

Ø Host range:

- ✓ Cultivated Solanaceae (potato, tobacco, tomato and pepper)
- ✓ Weeds



■ PVY in Tobacco

∅ 3 groups of isolates on *Nicotiana tabacum*

PVY^O



Mosaic

(Potato)

PVY^N



Necrosis

(Potato)

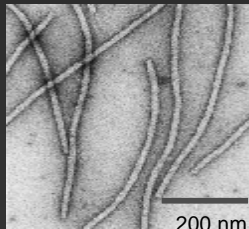
PVY^C



Mosaic

(Pepper, Tomato)

■ Control methods



Viral diseases are incurable

∅ Chemical control of vectors: ineffective

∅ Preventive control: cultural practices

∅ Genetic control: resistant cultivars





■ Genetic control of PVY in tobacco

∅ Recessive resistance *va* gene : 3 allelic forms *va*⁰, *va*¹, *va*²

Effect of *va*

Viral infection stage	Function	Literature
Multiplication	No effect	(Acosta-Leal, 2008)
Cell to cell movement	↓	(Acosta-Leal, 2008)
Necrosis symptoms	↓	(Verrier and Doroszewska, 2004)

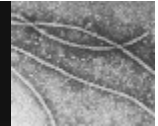
⇒ *va* gene introgressed in tobacco genotypes and grown in France since 3 decades. **However:** PVY infections described on *va*-derived genotypes



■ Question :

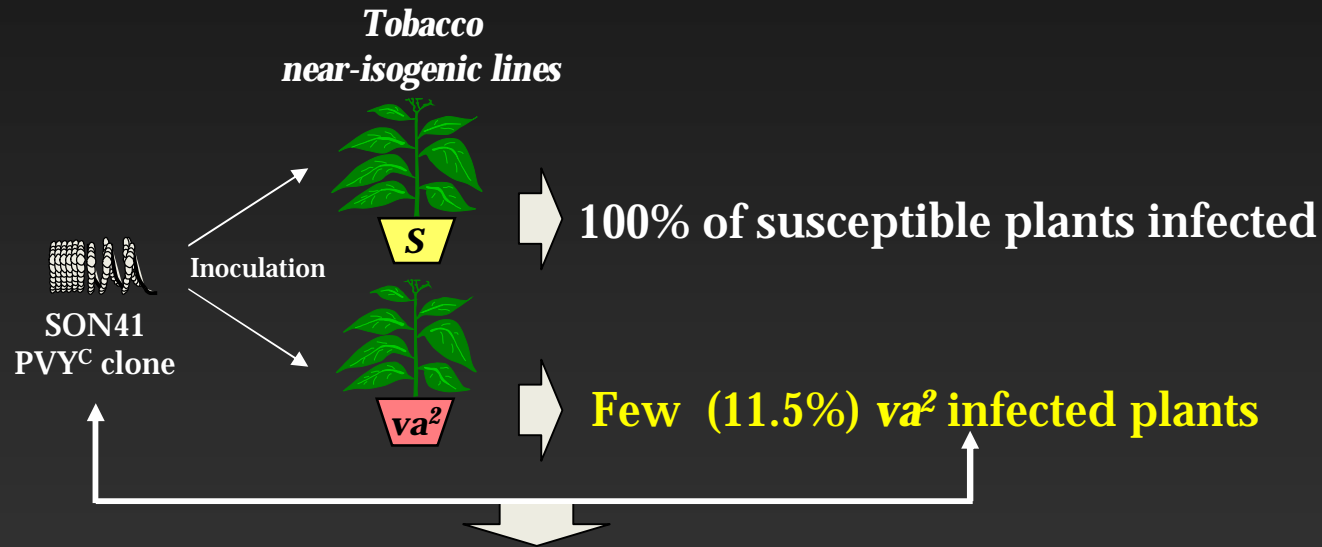
How the selective pressure imposed by the *va* alleles drive the evolution of PVY populations in tobacco?

Ø Impact on the development of strategies for durable resistance management

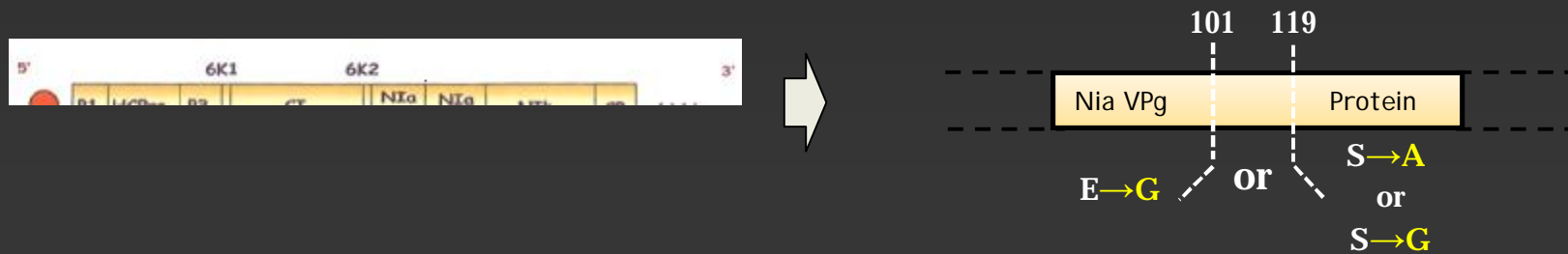


■ Highlighting adaptation of PVY to *va* allele

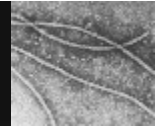
∅ Genetic adaptation of PVY^C infectious clone to the *va*² allele



Genetic comparison between initial SON41 clone and *va*² derived populations

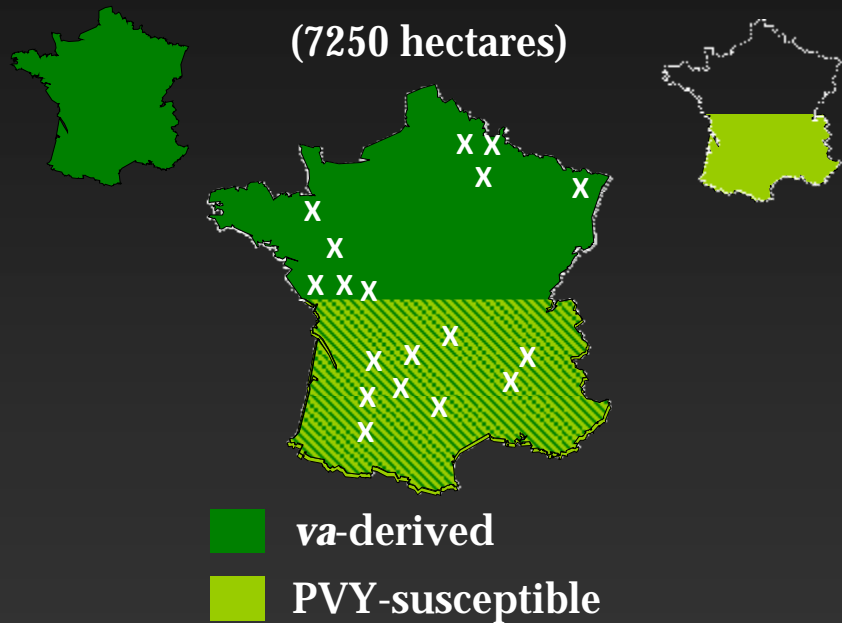


⇒ PVY adaptation to *va* requires one mutation in the VPg protein



■ Genetic polymorphism of VPg in PVY isolates

▼ Diversity of PVY in France: field survey in 2007



Tobacco genotypes

- **va-derived (allele 0 or 2)**
- **va-derived (allele 0 or 2) + PVY-susceptible**

x : Sampling areas: 556 leaf samples



Vein necrosis



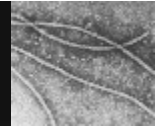
Mosaic



Unconventional symptom



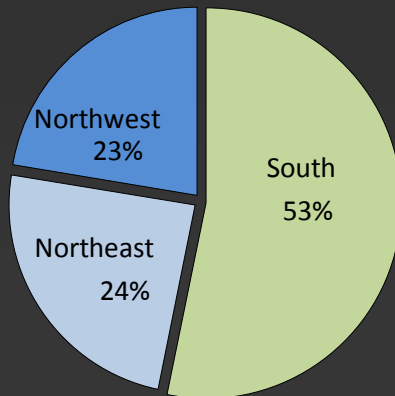
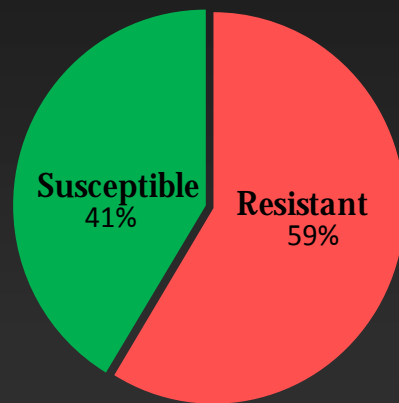
No symptom



■ Genetic polymorphism of VPg in PVY isolates

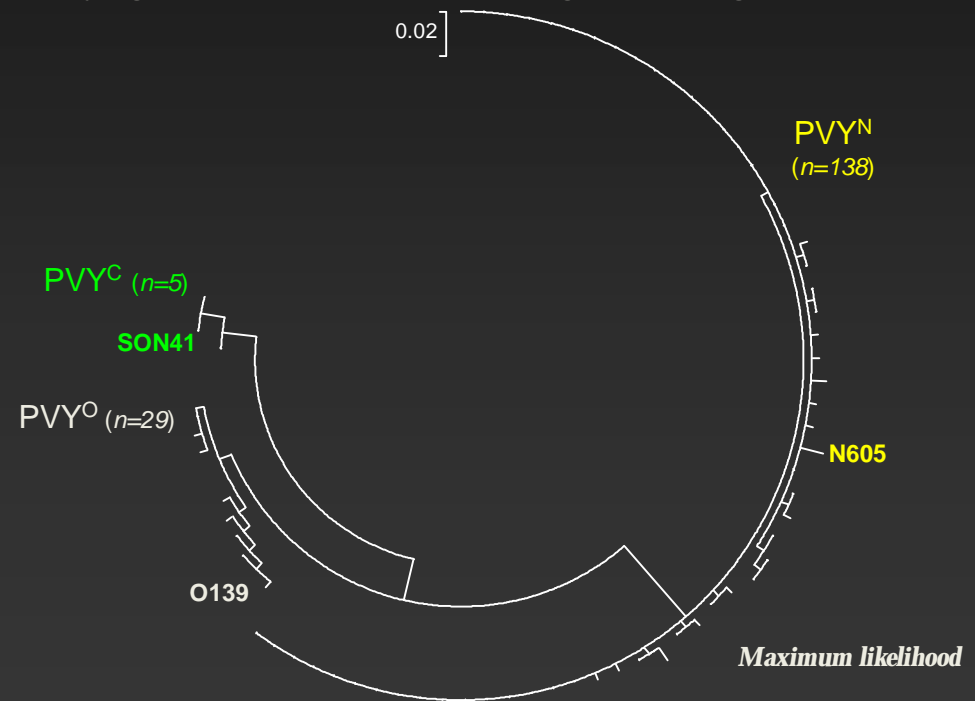
▼ Genetic polymorphism of VPg in sampled PVY isolates

201 PVY isolates analyzed

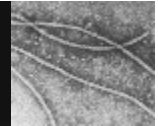


PVY phylogenetic tree

Phylogenetic tree obtained from VPg protein alignment



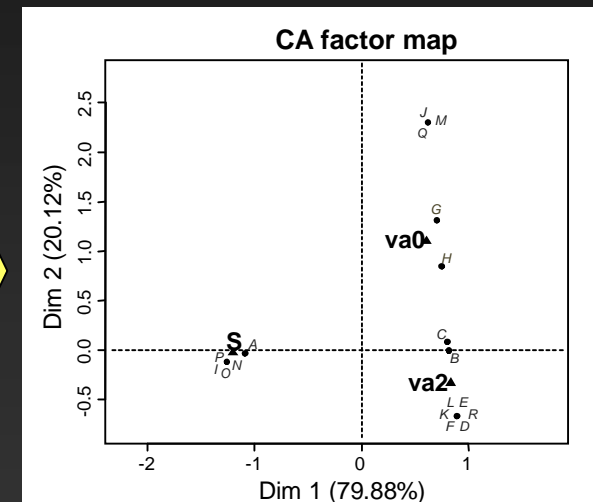
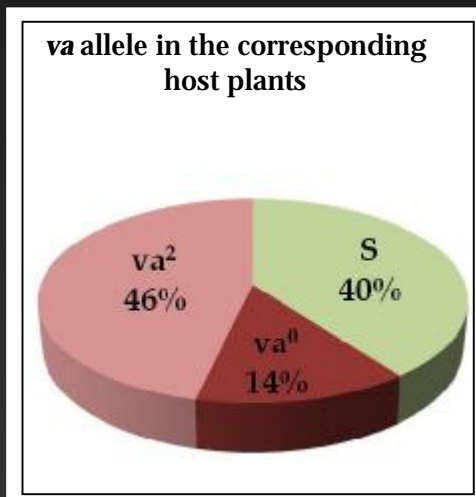
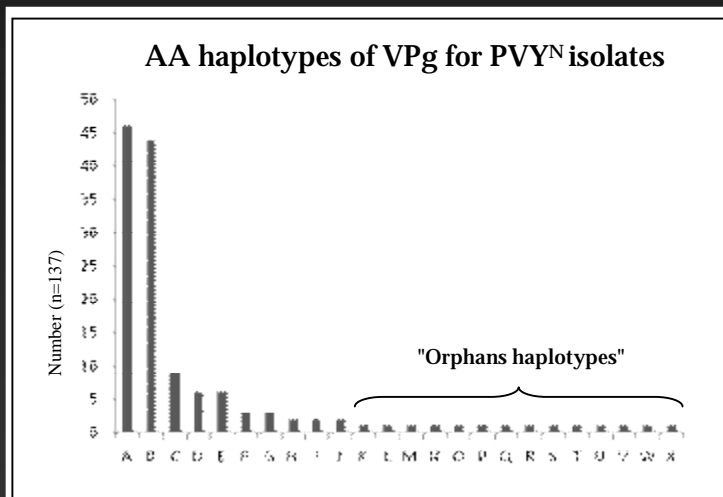
⇒ Isolates from the "N" group are
i) prevalent in populations and ii) poorly diversified



■ Correlation between VPg haplotypes and the *va* alleles

▼ Separated analysis for PVY^N and PVY^O:

▼ Example for PVY^N



⇒ Low variability at intra-group level with greater diversity in R tobaccos

⇒ Correspondence between VPg haplotypes and *va* resistance gene

VPg of PVY = viral avirulence factor



■ Detection of selection signature in PVY VPg

∅ AA under selection and comparative AA substitutions in S and R hosts

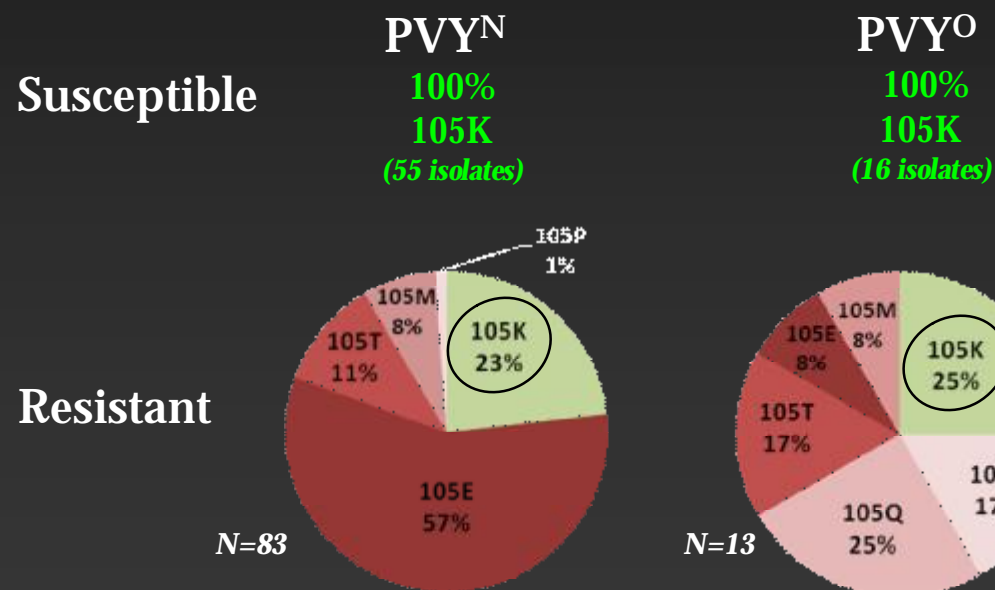
dN/dS analysis on the whole VPg encoding sequence (*SLAC in HyPhy*)



Only 1 codon detected under diversifying selection at position 105

Position already detected in PVY⁰ adaptation to VAM0 tobacco (*va⁰*)
Masuta & al. 1999

AA substitutions at position 105 in S vs. R hosts



⇒ VPg AA substitution for *va* adaptation seems occur preferentially at position 105 but other positions can be involved

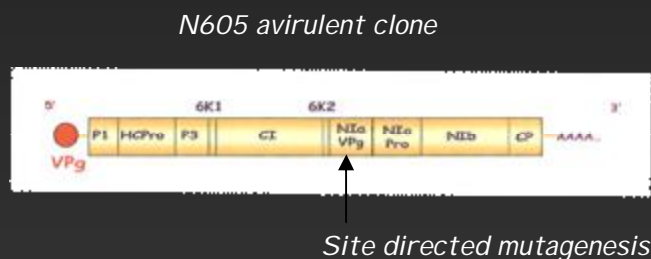


■ Evolutionary pathways for PVY adaptation

➤ Validation of the involvement of position 105 in PVY^N VPg for *va* adaptation

Introduction of mutations by directed site mutagenesis **PVY point-mutated clones** **Pathotype observed**

Virulence towards va alleles (VAM0, WISLI CA, PBD6) and sequencing



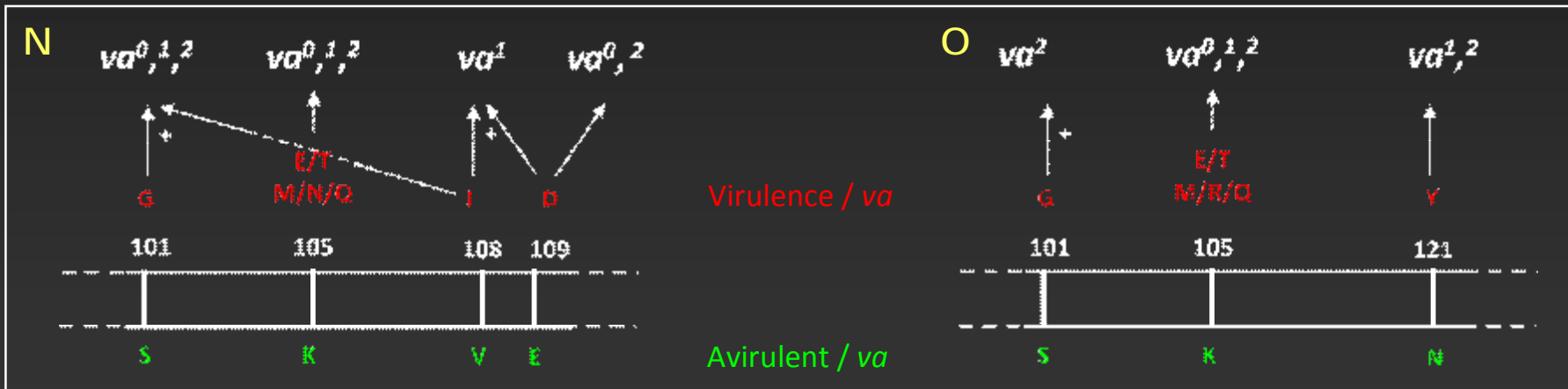
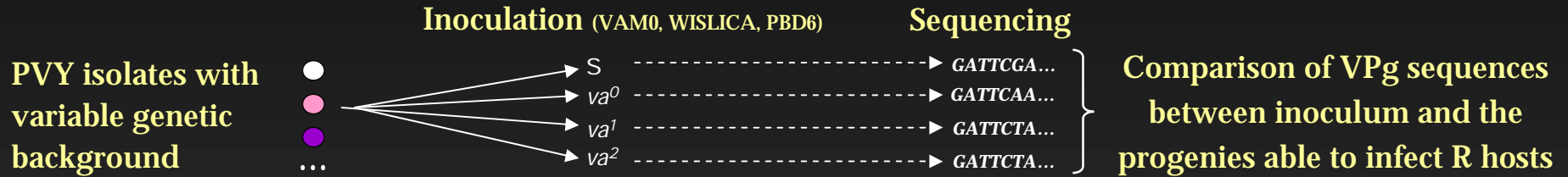
● → 105E	-----▶	0-1-2
● → 105M	-----▶	0-1-2
● → 105T	-----▶	0-1-2

⇒ The AA substitution at position 105 in the VPg of PVY^N is sufficient to confer adaptation to the different *va* alleles : *va*⁰, *va*¹ and *va*²



Evolutionary pathways for PVY adaptation

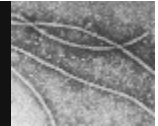
Ø Evolutionary pathway for PVY^N and PVY^O adaptation to *va*



⇒ Rapid evolution of PVY to adapt to *va*

⇒ *va* adaptation occurs preferentially by AA substitution at position 105

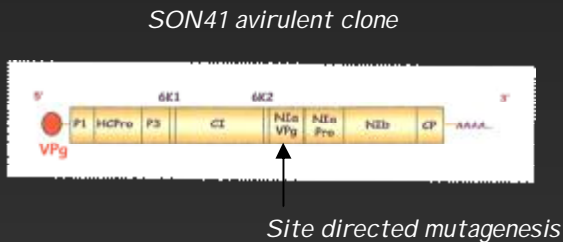
⇒ According to PVY genetic background other AA substitutions alone or in combination allow adaptation



■ Evolutionary pathways for PVY adaptation

➤ Validation of AA mutations in PVY^C VPg for *va* adaptation

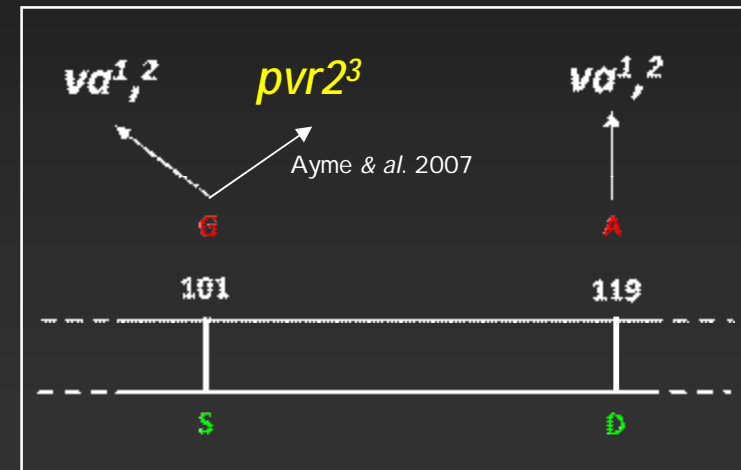
Introduction of mutations by directed site mutagenesis



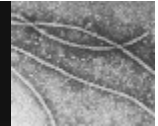
PVY point-mutated clones

- → 101G
- → 119A

Evolutionary pathway for PVY^C



⇒ Same AA substitution at position 101 confer together adaptation to the *va*^{1,2} alleles in tobacco and to *pvr2*³ resistant allele in pepper => **Cross-Virulence !**



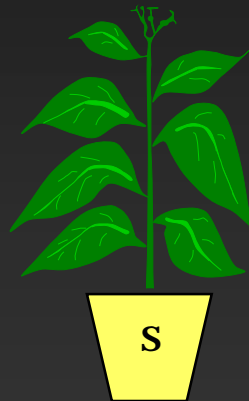
■ Prospects: Competitiveness of *va* adapted mutants

∅ Estimation of fitness cost associated with adaptive mutations

Competitions between :

Avirulent
and
Virulent clones

Into susceptible plant



Inoculation at J-0

Monitoring of the relative ratio in inoculated plants

Avirulent vs. **virulent** populations



=> Estimation of fitness cost associated with adaptive mutations

=> Prediction of the maintenance of PVY virulent isolates in field populations

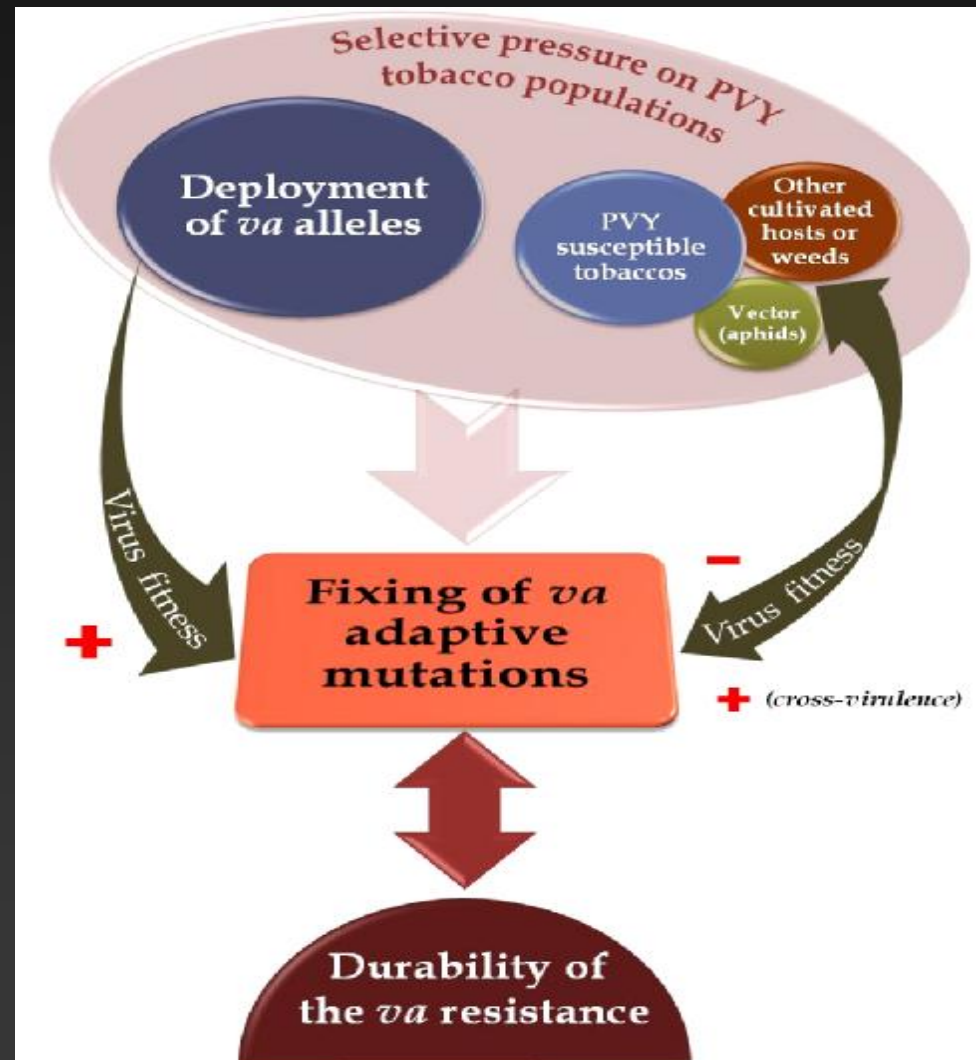
■ Strategies for durable management of the *va* resistance

∅ Deployment of *va* alleles in commercial tobacco cultivars

- Rapid evolution of PVY to adapt to *va*
- Same mutation confer virulence to all *va* alleles => Turn-over impaired
- Take into account environment :
...as cross-virulence phenomenon

∅ According to fitness cost :

- Reduction of the selective pressure in field
- Increase fitness cost associated to the gain of virulence (QTLs)





■ Conclusions

PVY populations on tobacco are characterized by:

- Large proportion of PVY^N isolates in PVY population but low variability at intra-group level
- In susceptible host, PVY diversity seems lower than in resistant hosts

Rapid adaptation of PVY to *va* which requires:

- Only one infection cycle on a resistant host
- A single amino acid substitution in the central part of the VPg protein (*preferentially in position 105*)

Depending on PVY genetic background different amino acid substitutions in the VPg protein could confer adaptation to some *va* alleles

Durable management of the *va* resistance depends on VPg evolutionary constraints => selective pressure imposed by *va* and the environment

Acknowledgments

INRA, Rennes Team “Biology and evolution of plant RNA viruses”

C. Lacroix

E. Jacquot

L. Glais

M. Tribodet

A. Delaunay

I. Abt

M. Guillet

F. Faurez

G. Riault

R. Mabon

F. Boulard



INRA, Avignon

B. Moury

Association for research on *Nicotianae* Institut du tabac Bergerac Imperial Tobacco group

E. Hirsch

Y. Saint-Jalm

B. Vidal

W. Roeper

J.L. Verrier

B. Cailleteau