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# **Characterization of PVY (Potato Virus Y) resistance in tobacco: potential role of an eIF4E gene identified by high throughput sequencing technologies.**

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## Impact

- The potyvirus PVY is worldwide spread, particularly in Europe or South America with aggressive necrotic strains. This virus is transmitted by aphids.
- Symptoms can be vein banding, vein clearing, chlorotic spots, necrosis: loss of yield and quality.

## Resistance sources

- *N. tabacum* deletion: different allelic form of « va » recessive gene.
- *N. africana* (Lewis 2005, 2007).



## Limits of va resistance

- Adverse effects of va gene observed on tobacco quality or leaf surface exudates (Nogushi 1999; Nielsen 1982).
- Appearance of resistance breaking variants.

## Interaction between VPg and eIF4E



- Viral RNA has a 5'-genome linked protein (Vpg) and a 3' polyadenylated tail.



- VPg is able to interact with eukaryotic initiation factor (eIF4E) in host plant.
- Viruses recessive resistance factors in many plants have been identified as eIF4E genes.

Organism	Lettuce	Melon	Tomato	Pepper	barley	Pea
Gene	Mo1	Nsv	Pot-1	Pvr1, pvr2, pvr6	Rym4, rym5, rym6	Sbm1, wlv, cyv2

- Such interaction has not been shown in tobacco yet.

# Methodology to identify candidate resistance genes



Population: F7  
Recombinant Inbred Lines

5 lines PVY<sup>R</sup>

No symptoms



7 lines PVY<sup>S</sup>

Symptoms

12 mRNA libraries

Illumina Hi-Seq-2000



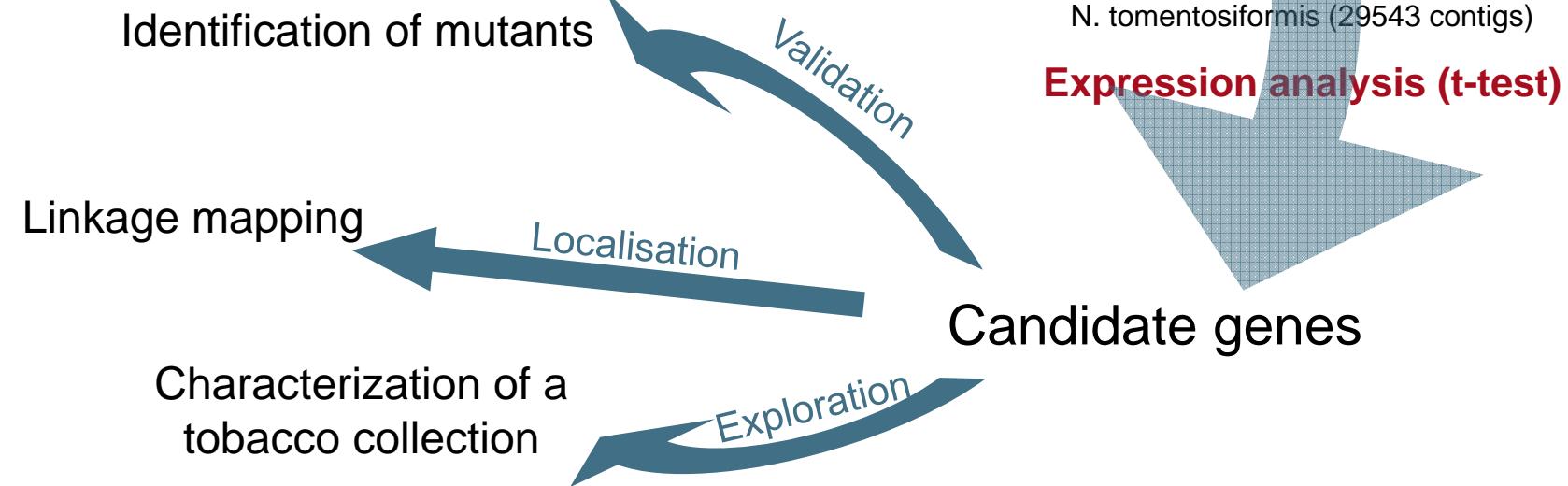
CLC Genomics Workbench

**Mapping on reference transcriptomes:**

N. sylvestris (32852 contigs)

N. tomentosiformis (29543 contigs)

**Expression analysis (t-test)**



## Differential expression between S and R lines: t-test



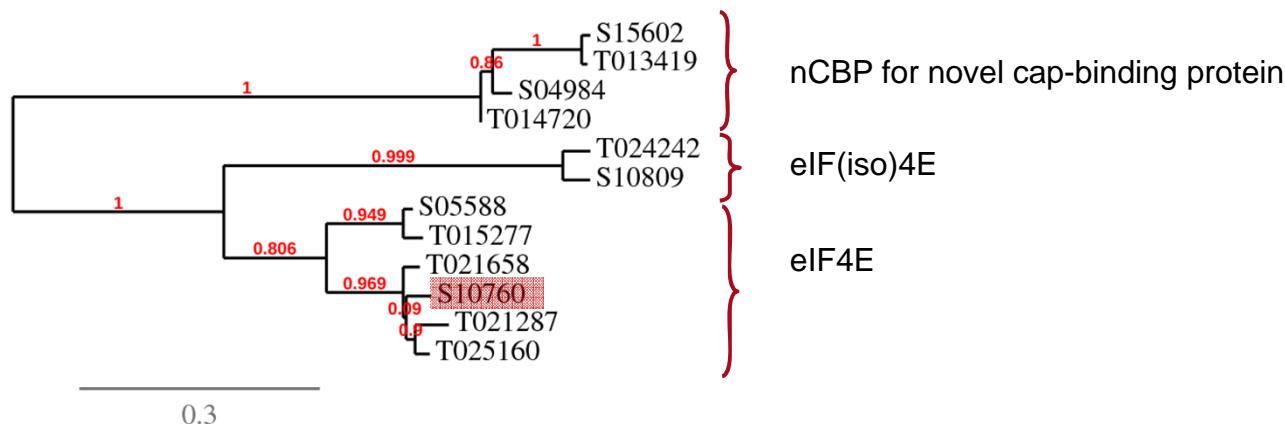
Contig ID	t-test		expression values		Annotation
	Test statistic	P-value	R-means	S-means	
S21268	33,0	1,5E-11	1,1	71,1	3-isopropylmalate dehydratase, putative
T006855	30,6	3,2E-11	2,7	4,8	hypothetical protein MTR_8g036520
S28892	26,4	1,4E-10	2,0	25,0	JHL07K02.14
S23375	22,3	7,3E-10	5,5	292,1	chlorophyll a/b-binding protein Cab-1A
S32668	21,4	1,1E-09	7,5	285,3	chlorophyll a/b binding protein-like protein
S29811	20,1	2,0E-09	0,2	8,3	hypothetical protein MTR_4g113100
S23702	19,7	2,5E-09	29,0	1043,2	putative chloroplast chlorophyll A-B binding protein
S07708	19,5	2,7E-09	0,0	5,9	heat shock factor
S22103	19,4	3,0E-09	42,4	196,6	chlorophyll a/b binding protein-like protein
S32718	18,5	4,6E-09	3,2	118,8	chlorophyll a-b binding protein 50
S24839	18,1	5,8E-09	20,5	76,8	conserved hypothetical protein
S29023	18,0	5,9E-09	10,8	535,6	-
<b>S10760</b>	<b>17,9</b>	<b>6,2E-09</b>	<b>1,0</b>	<b>22,6</b>	<b>eukaryotic translation initiation factor 4E</b>
S32736	16,2	1,7E-08	5,8	258,6	-
S02317	15,9	2,0E-08	0,1	3,2	NRC1
S23035	15,9	2,0E-08	5,6	76,1	chlorophyll a/b binding protein

Contig S10760 is an eIF4E gene preferentially expressed in susceptible lines !

## Characteristics of contig S10760



- High homology with 12 eIF4E genes identified in tobacco



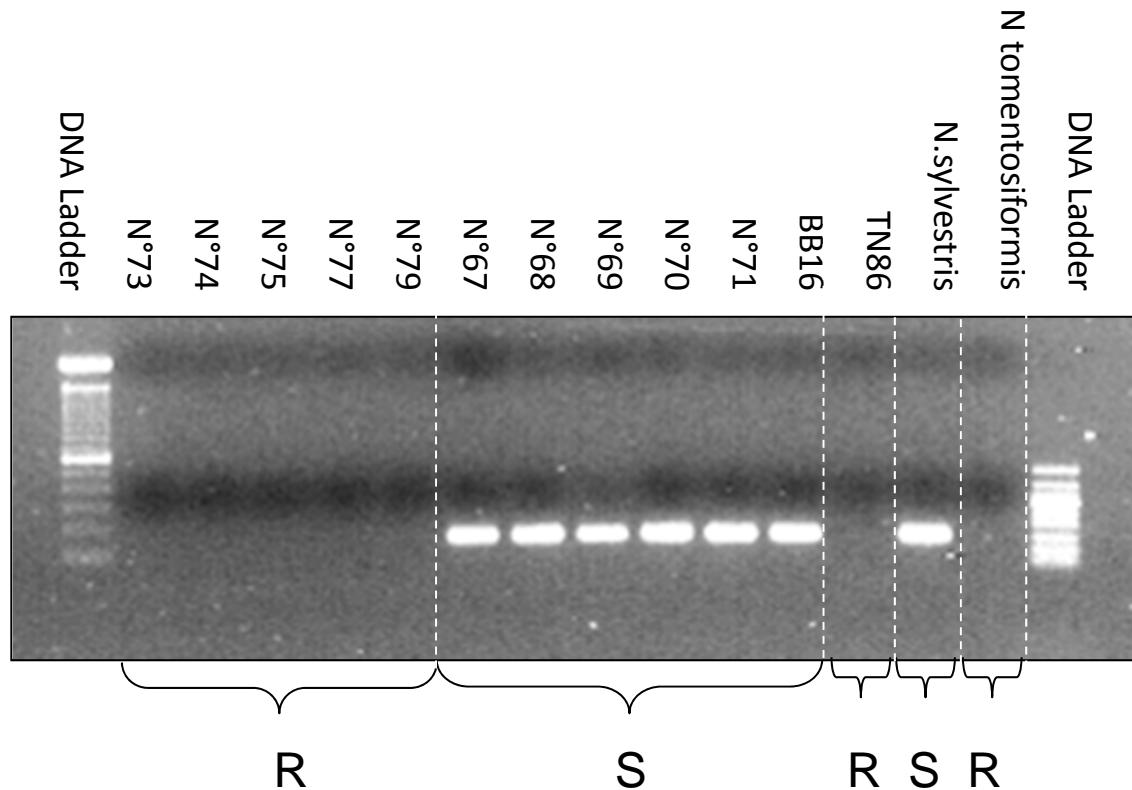
- S10760 = 1020 bp length, 5 exons (Genbank released under accession KF155696)



- 84% homology with pepper and tomato protein

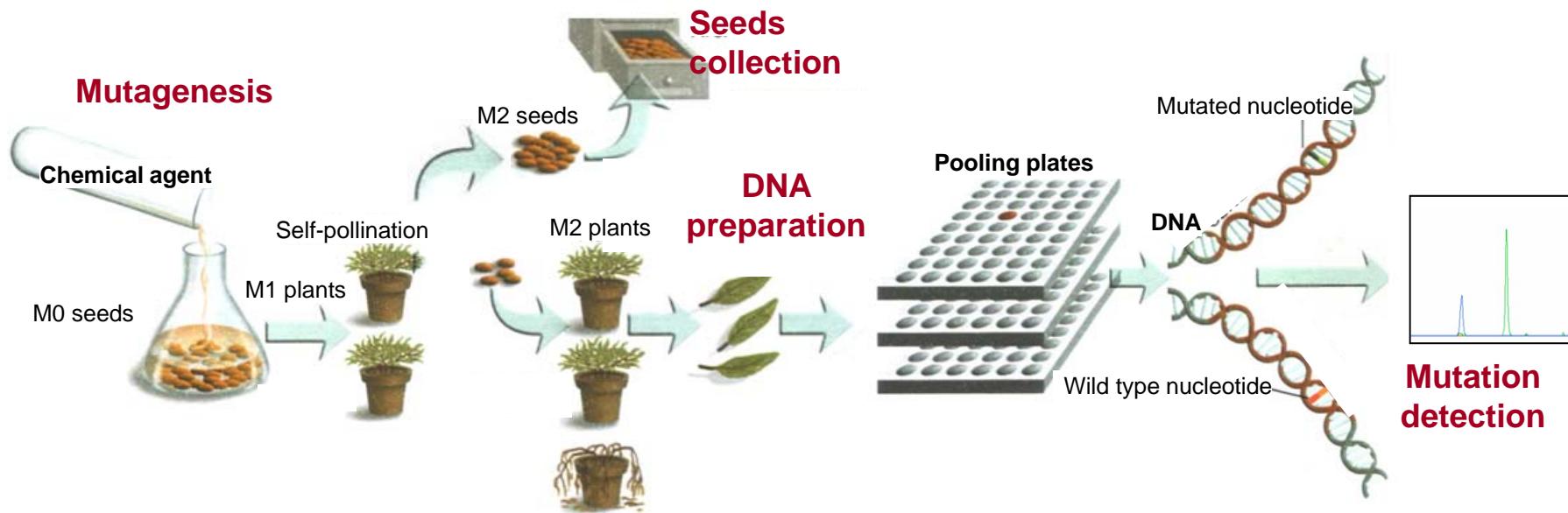
	10	20	30	40	50	60	70	80	90	100	110
Nicotiana	-	-MAEAEKILRV--DEVEVADGPEEGIEIVDESDDTASYLGKEIKPKHPLENSWTFWFDNPMAKS	RQAAWGSLLRELYTFSTIVEDFWGVYNNN	HPSKL	VV	GADLHC					
Capsicum	MATAEMEKTTFDEAEKVKL--NANEAD	DEVEEGIEIVEETDDT	TSYLSKEIATKHPLEHSWTFWFDNPVAKS	KQAAWGSLLRN	VYT	FSTVEDFWGAYNNI	HPSKL	VV	GADLHC		
Solanum	MAAAEMERTMSFDAAEKILKAADGGGGEV	DDELEE	GIEIVEESNDTASYLGKEITV	KHPLEHSWTFWFDNP	TTKS	RQAAWGSLLRN	VYT	FSTVEDFWGAYNNI	HPSKL	IMGADLHC	
	126	136	146	156	166	176	186	196	206	216	226
Nicotiana	KHKIEPKWEDPV	CANGGNW	TMSFSKGKSDTSWLYTLLAMIGHQFDHGEE	ICGAVVSVRN	GDKIALWT	KNAANETAQVSIGKQWKFLDYSNS	IGFIFH	-	-	-	-
Capsicum	KHKIEPKWEDPV	CANGGT	WMSFSKGKSDTSWLYTLLAMIGHQFDHEDIE	ICGAVVSVRGKE	KISLWT	KNAANETAQVSIGKQWKFLDYSNS	VGFV	FHD	AKRLDRNAKNRY	TV	
Solanum	KHKIEPKWEDPV	CANGGT	WMSFSKGKSDTSWLYTLLAMIGHQFDHGEE	ICGAVVSVRA	KEGKIALWT	KNAANETAQVSIGKQWKFLDYSNS	VGFV	FHD	AKRLDRNAKNRY	TV	

## DNA amplification of contig S10760 in resistant and susceptible RILs and controls



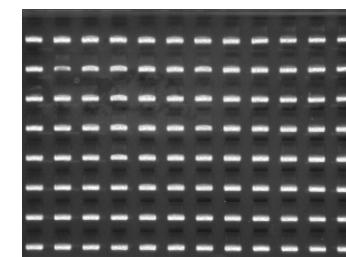
- Contig S10760 is present in susceptible plants
- No amplification in resistant plants = eIF4E gene deleted.

# Validation on tobacco mutants

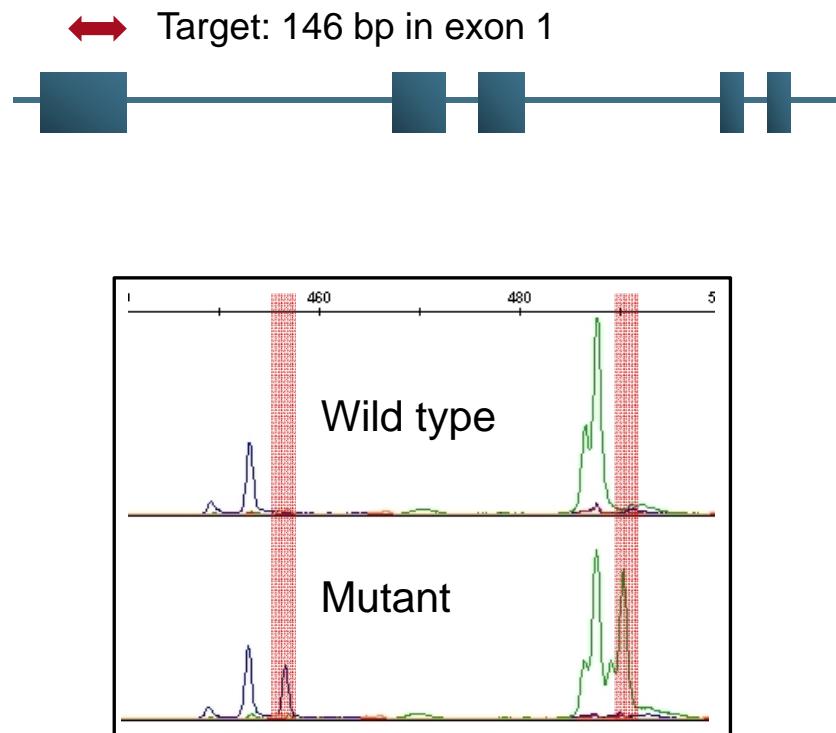


Original population is susceptible to PVY

(Science et Vie, 2002)



# Detection of eIF4E variants



Detection of mutants by Capillary-Electrophoresis Single Strand Conformation Polymorphism

Mutant Id.	Mutation	Amino-acid change	SIFT score
E3-289	G/A	E47K	0,1
E3-1097	<b>G/A</b>	<b>W50*</b>	<b>0</b>
E3-1198	G/A	E47K	0,1
E3-929	C/T	P57S	1
E2-217	G/A	W53*	0
E1-292	C/T	A64V	0,31
E1-39	C/T	A33V	0,29
E1-74	C/T	T32M	0,23
E1-167	C/T	A33V	0,29
E1-216	G/A	R62K	1

Ten mutants were obtained in exon 1, including two stop codon.

## Biological test for PVY resistance on mutants: example of E3-1097 mutants carrying a stop codon



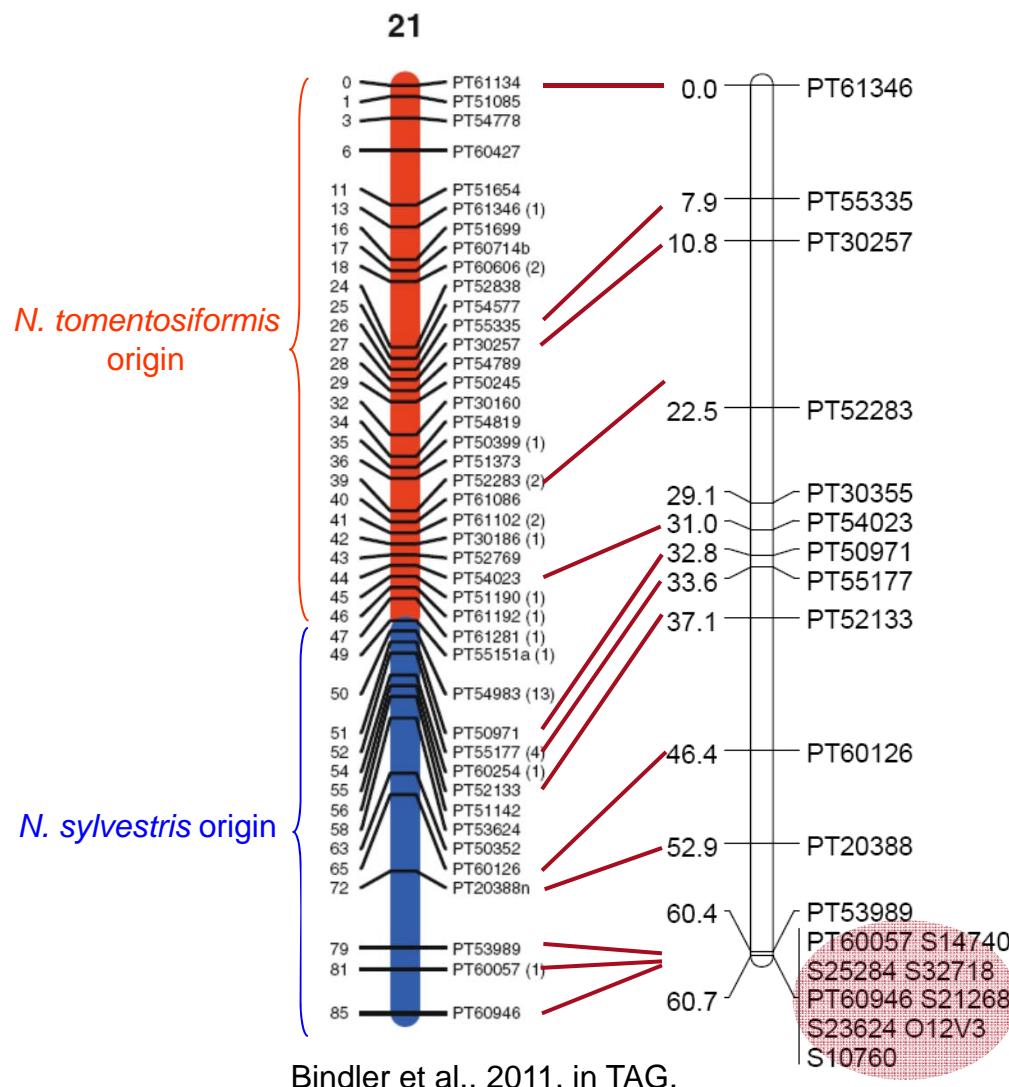
M2 mutants were genotyped by CE-SSCP and characterized for PVY resistance.

Genotyping		Wild type	Mutant	Heterozygous	Total	P
Bio. Test	Susceptible	22	0	42	64	0.46
	Resistant	0	15	0	15	
	Total	22	15	42	79	

Chi2 test for ratio of wild type, heterozygous and mutants in M2 mutants families. P>= 0.01 indicates that segregation in the observed population does not differ significantly from the expected ratio ( $\frac{1}{4}$  W;  $\frac{1}{4}$  M and  $\frac{1}{2}$  H).

- Complete linkage between mutated eIF4E gene and PVY resistance.

# Linkage mapping of eIF4E and others differentially expressed genes



- Genetic mapping in a F2 population segregating for PVY resistance.
- eIF4E and other differentially expressed genes colocalize on *N. sylvestris* arm of chromosome 21.
- O12V3 marker linked to « va » resistance colocalizes too.

O12V3 = marker of va gene (Noguchi et al., 1999).

S14740, S25284, S32719, S21268, S23624 = candidate genes differentially expressed between S and R lines (t-test table).

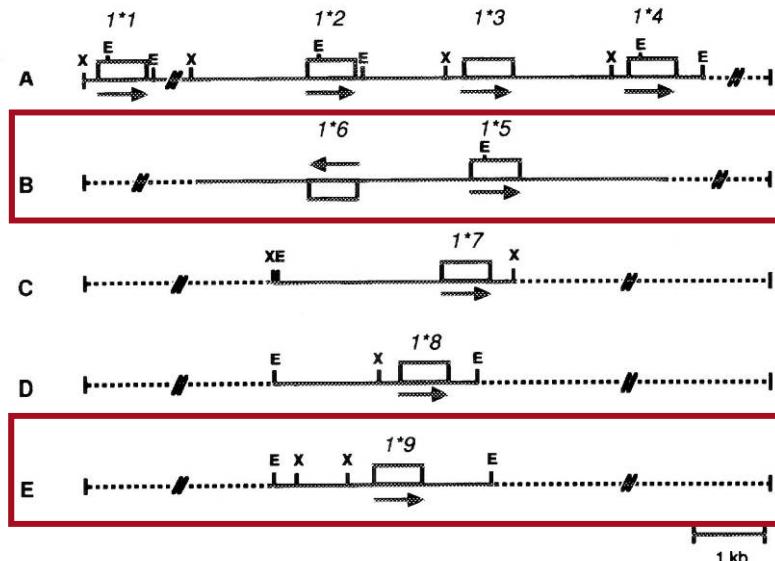
This group of markers is absent in resistant plants.

# Differential expression between S and R lines: Ihcb genes are preferentially expressed in susceptible lines.

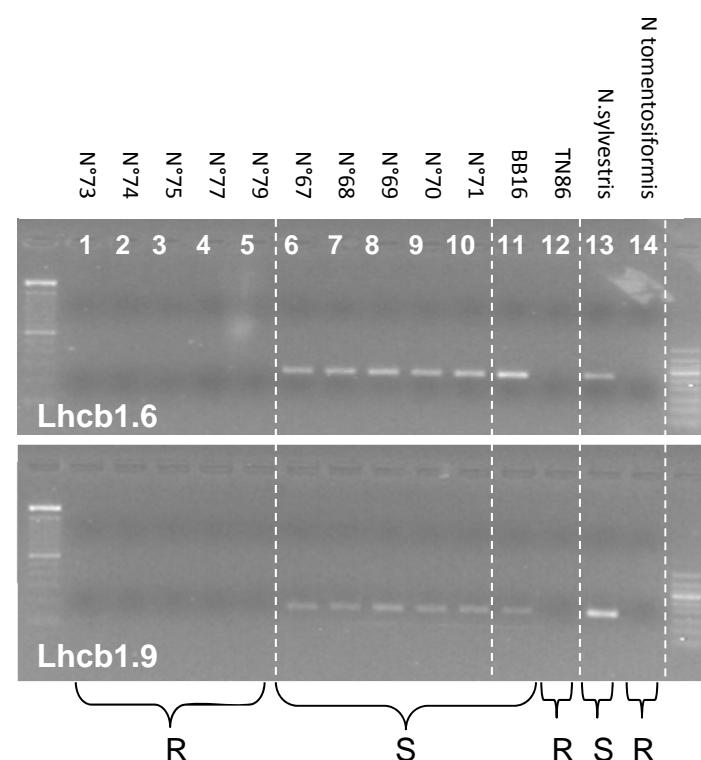


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# Organization of Lhcb1 genes in *N. sylvestris*



Nine Lhcb1 genes encoding for chlorophyll a/b binding protein have been identified in *N. sylvestris* (Hasegawa et al. 2002, Gene)



- Deletion of Lhcb1.5, Lhcb1.6 and Lhcb1.9 in *N. tabacum* PVY resistant plants.

# Characterization of 163 tobacco varieties



Tobacco type	Susceptible A	Resistant			Total
		B	C	D	
Kentucky/Maryland	12	1	0	0	13
Burley	14	9	0	1	24
Dark Air-Cured	40	11	2	4	57
Flue-Cured	32	7	2	6	47
Oriental	14	0	0	2	16
Experimental	6	0	0	0	6
Total	118	28	4	13	<b>163</b>



PCR amplification on :

- ✓ eIF4E
- ✓ contigs S21268, S14740, S23035, S23624, S32718 (differentially expressed)
- ✓ O12V3 (linked to va).

A= Susceptible varieties, all the contigs are present.

B= Resistant varieties, all the contigs are absent.

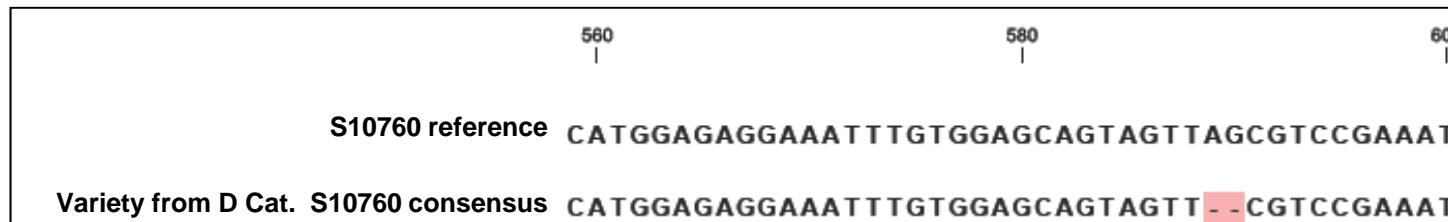
C= Resistant varieties, eIF4E is absent but others contigs are present: small deletion around eIF4E.

D= Resistant varieties, eIF4E and others contigs are present.

## Plants from D category (eIF4E present)

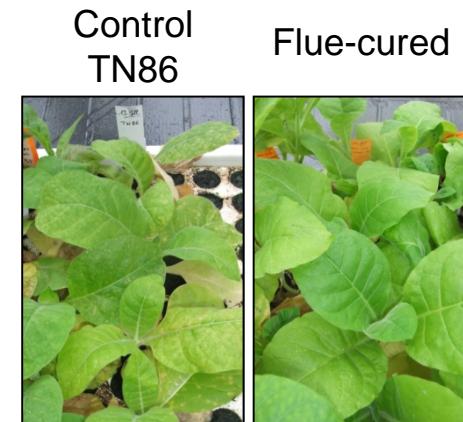


1. One 2bp deletion in eIF4E gene, conferring resistance to PVY (observed in 3/13).



2. Another gene could be involved (10/13).

- Better resistance against PVY resistance breaking variants (mutation on amino-acid 105 of the Vpg, Janzac et al., personal communication).
- F2 crosses segregating for PVY resistance are under investigation with this resistance.
- RNA-Seq on these plants will be investigated too.



## To conclude



- Illumina transcriptome sequencing has proven high efficiency for differential expression analysis, coupled with mutagenesis to validate candidate gene.
- Tobacco PVY susceptibility involves an eIF4E gene.
- Deleterious effects observed with classical va deletion could be limited by using varieties with a small deletion in eIF4E. To be investigated...
- Combination of different sources of resistance could help to fight against PVY resistant breaking variants.

## The team



Thanks to Thierry Candresse  
from INRA Bordeaux



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Imperial Tobacco Bergerac



Francois  
Dorlhac



Emilie  
Julio



Julien  
Cotuchateau



Christophe  
Decrops



Roxane  
Volpatti