

# 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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# PVY Characteristics

## 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

- 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
<b>Gene</b>	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)**

Identification of mutants

Validation

Linkage mapping

Localisation

Characterization of a tobacco collection

Exploration

Candidate genes

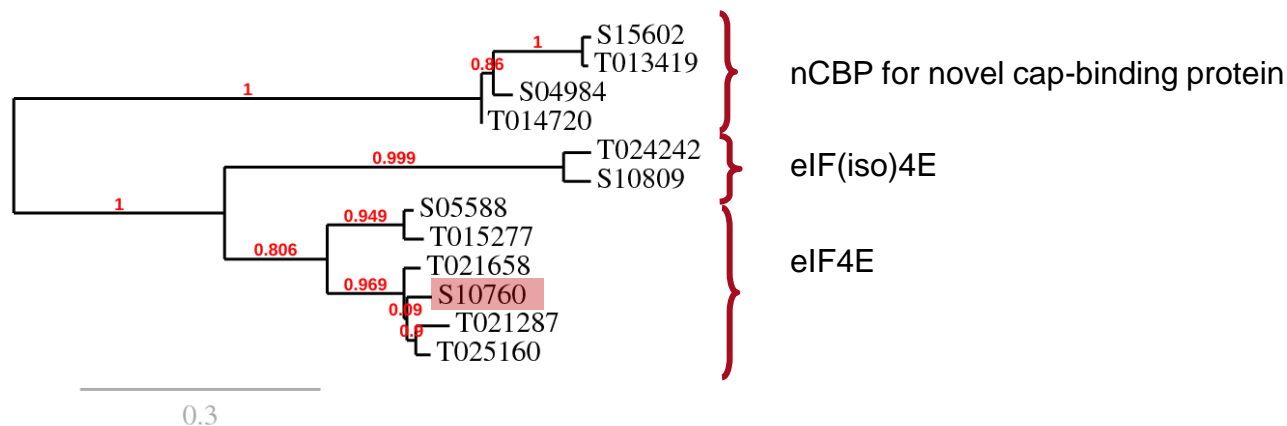
# Differential expression between S and R lines: t-test

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 expressed in susceptible lines**

# Characteristics of contig S10760

- High homology with 12 eIF4E genes identified in tobacco



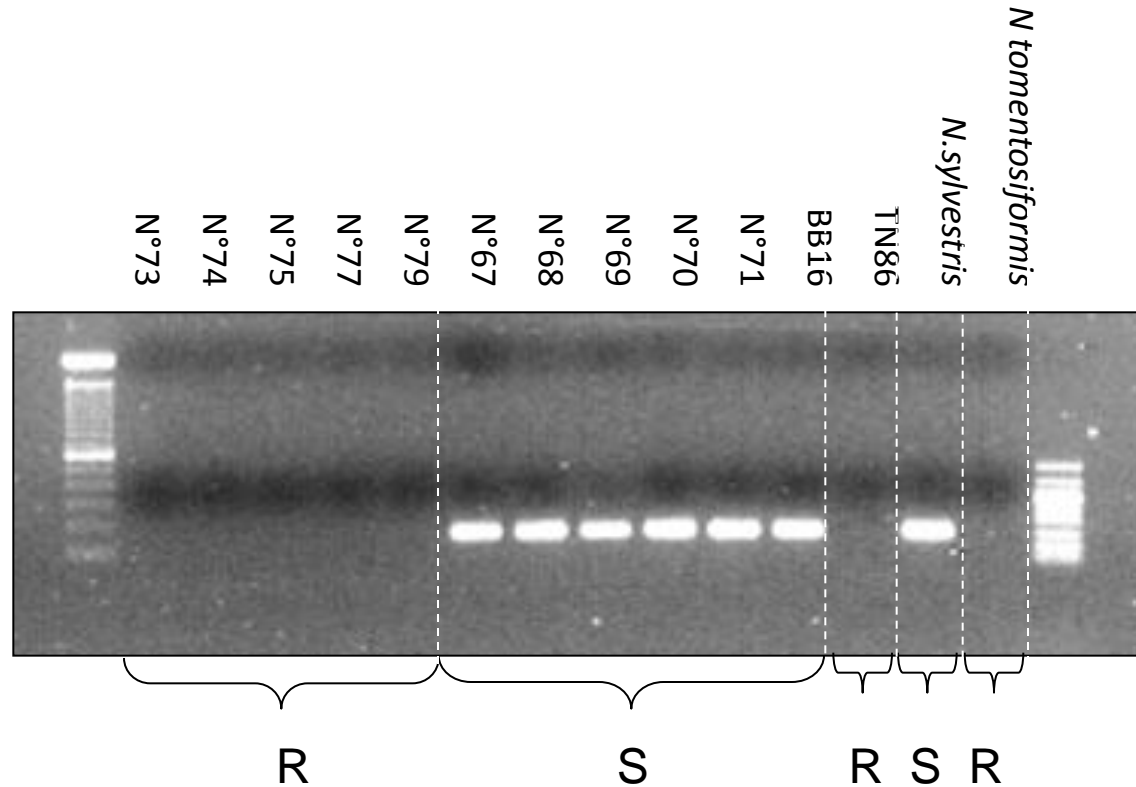
- S10760 = 1020 bp length, 5 exons (released on Genbank under accession KF155696)



- 84% homology with pepper and tomato protein

	10	20	30	40	50	60	70	80	90	100	110
Nicotiana	-----MAEAEKLRV--DEVEVADDGPEEGEIVDESDDTASVYLGKEIKPKHPLENSWTFWFDNPMAKSRQAANGSSLRLRLYTFSTIVEDFWGVYNNINHPKLVVGGADFHCF										
Capsicum	MATAEMKTIITFDEAEKVKL---NANEADDEVEEGEIVEETDDTTSYLSKEIATKHPLEHSWTFWFDNPMVAKSKQAANGSSLRNVTFTSTIVEDFWGAYNNIHHPSKLVVGGADLHCF										
Solanum	MAAAEMERTMSFDAAEKIKAADGGGGEVDEDEEAGEIVEESNDTASVYLGKEITVKHPLEHSWTFWFDNPTTKSRQTANGSSLRNVTFTSTIVEDFWGAYNNIHHPSKLVVGGADFHCF										
	126	136	146	156	166	176	186	196	206	216	226
Nicotiana	KHKIEPKWEDPVCANGGNWIMSFSGKSDTSWLYTLLAMIGHQFDHGEDEICGAVVSVRNKGDKIALWTKNAANETAQVSIQKQWKEFLDYSNSIGFI FH-----										
Capsicum	KHKIEPKWEDPVCANGGIWRMSFSKGSKSDTSWLYTLLAMIGHQFDHDEDEICGAVVSVRKGKIKISLWTKNAANETAQVSIQKQWQFLDYSDSVGFIFHDDAKRLDRNARNRYTV										
Solanum	KHKIEPKWEDPVCANGGIWRMSFSKGSKSDTSWLYTLLAMIGHQFDHDEDEICGAVVSVRAKGEKIALWTKNAANETAQVSIQKQWQFLDYSDSVGFIFHDDAKRLDRNARNRYTV										

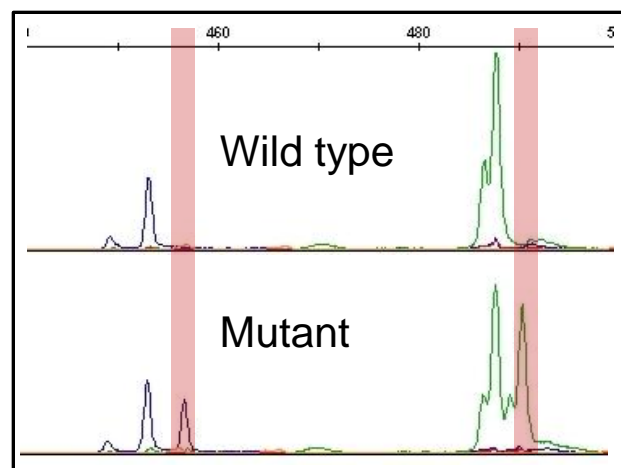
# DNA amplification of contig S10760 in resistant and susceptible RILs and controls



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

# Detection of eIF4E mutants in tobacco

↔ Target: 146 bp in exon 1



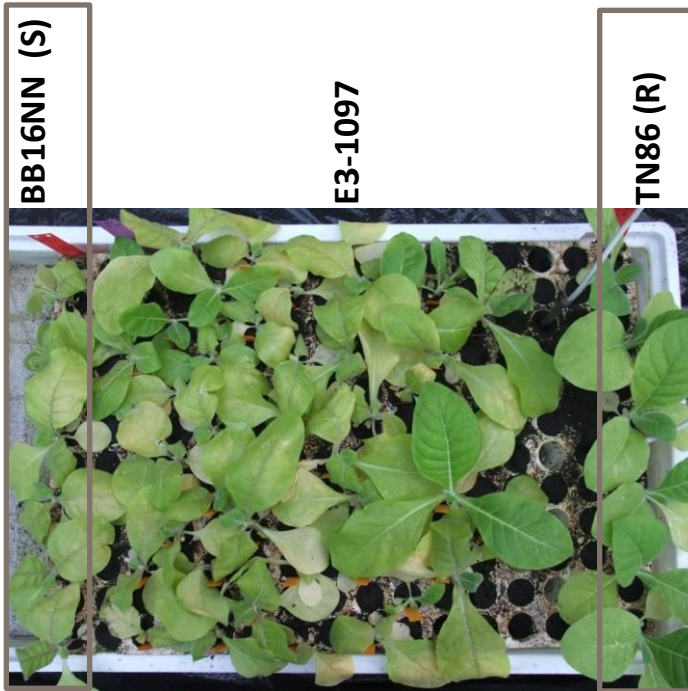
Detection of mutants by Capillary-Electrophoresis Single Strand Conformation Polymorphism.

Mutant Id.	Mutation	Amino-acid change	SIFT score
E3-289	G/A	E47K	0,1
E3-1097	G/A	W50*	0
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



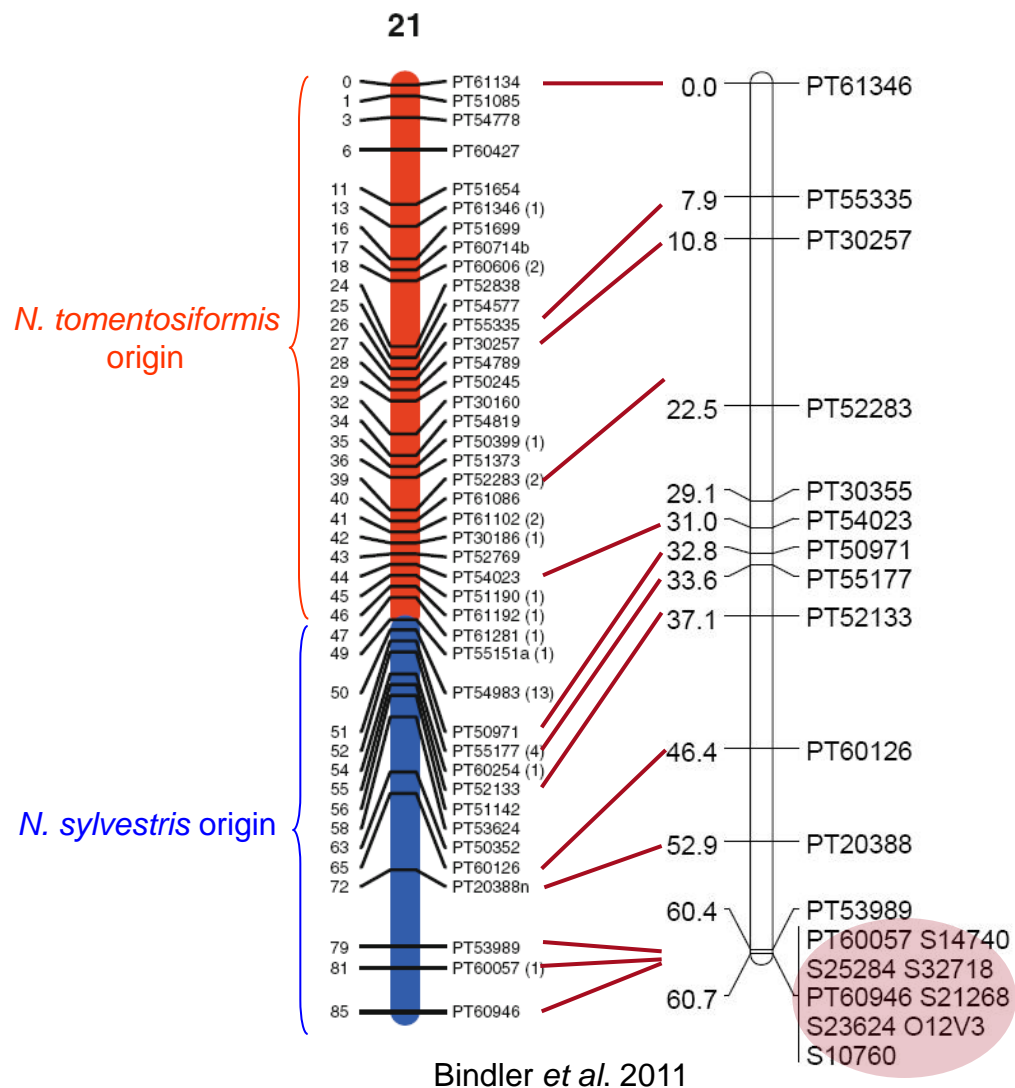
M2 mutants were genotyped by CE-SSCP and characterized for PVY resistance.

		Wild type	Homo zygous	Hetero zygous	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.  $P \geq 0.01$  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 other differentially expressed genes



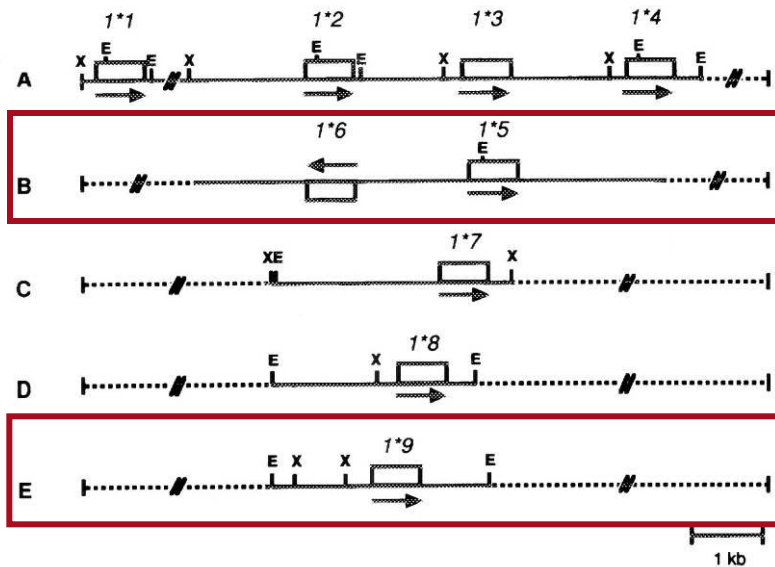
- Genetic mapping in a F2 population segregating for PVY resistance.
- eIF4E and other differentially expressed genes colocalize on *N. sylvestris* arm of chromosome 21.
- O12V3 marker linked to « va » resistance colocalizes too.

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

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

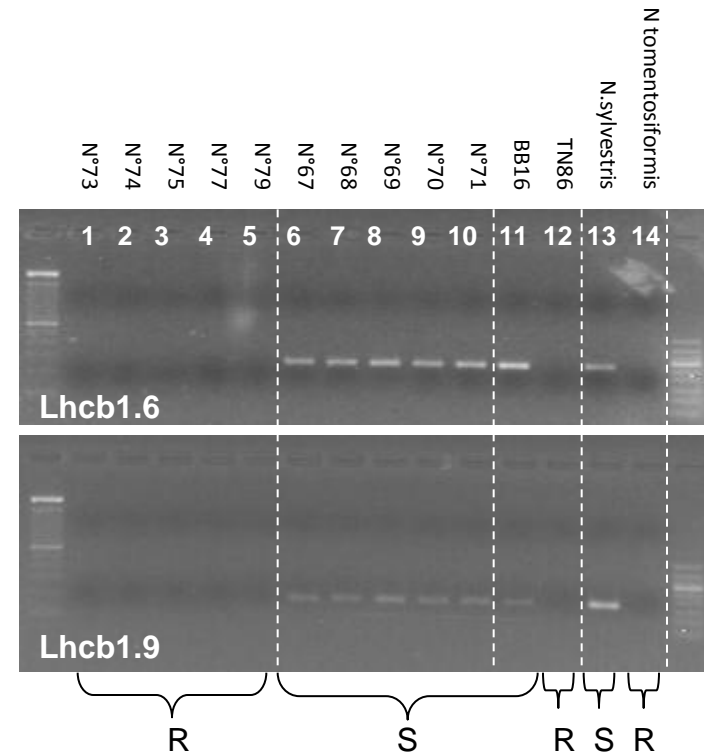
This group of markers is absent in resistant plants.

# Three lhcb genes are absent



- Lhcb1.5, Lhcb1.6 and Lhcb1.9 are absent in *N. tabacum* PVY resistant plants.

Nine lhcb1 genes encoding for chlorophyll a/b binding protein have been identified in *N. sylvestris* (Hasegawa *et al.* 2002).



# Characterization of 157 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
<b>Total</b>	<b>112</b>	<b>28</b>	<b>4</b>	<b>13</b>	<b>157</b>



A = Susceptible varieties, all the contigs are present 👍

B = Resistant varieties, all the contigs are absent 👍

C = Resistant varieties, eIF4E is absent but the other contigs are present 👍

D = Resistant varieties, eIF4E and the other contigs are present ?

## Plants from D category (eIF4E present)

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

	560		580		600
S10760 reference	CATGGAGAGGAAATTTGTGGAGCAGTAGTTAGCGTCCGAAAT				
Variety from D Cat. S10760	CATGGAGAGGAAATTTGTGGAGCAGTAGTT - - CGTCCGAAAT				

2. Another gene could be involved (10/13).
  - High resistance against PVY breaking variants.
  - F2 crosses segregating for PVY resistance are under investigation.
  - These plants will be studied by RNA-seq too.

## To conclude



- RNAseq and mutagenesis is probably the best combination of tools to identify resistance genes yet.
- The va gene is an eIF4E.
- Using variants with a small deletion in the eIF4E gene could help to limit the loss of quality.
- A new source of resistance is under investigation to fight against PVY breaking variants.



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