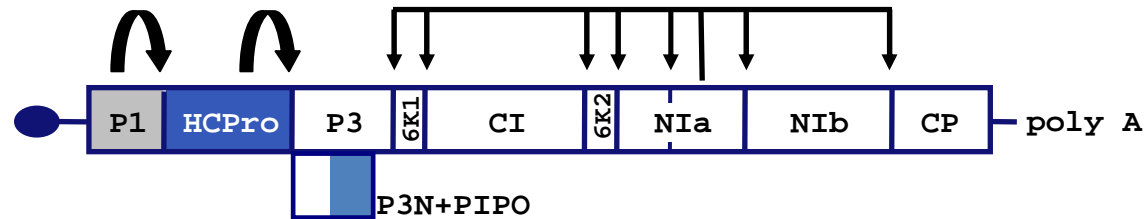


**P1 and CP, the two ends of the potyviral
polyprotein, are involved in viral pathogenicity
and host adaptation**

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Mateo

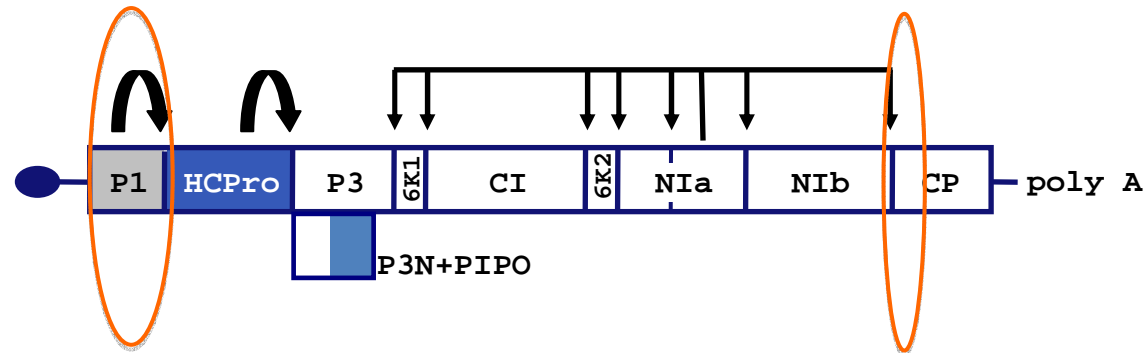
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Plum pox virus (sharka virus, PPV)



- Family *Potyviridae*, genus *potyvirus*
- Single-stranded RNA molecule of 10 kb
- Expressed through a large potyprotein that is subsequently processed into at least 10 mature proteins
- Infects trees of the genus *Prunus* and herbaceous plants

? The capacity of PPV to establish local and systemic infections in woody and herbaceous hosts has not yet been accurately located



P1 is the most variable protein among different potyviruses :
Host-specific interactions??

- 2 mutations have been identified that are associated with mild pathogenicity of some subisolates of the M-type PPV-PS isolate

N terminus of the **CP**: high variability in length and sequence

- contribution of particular sequences to **specific adaptation** to herbaceous and woody hosts has been shown for PPV

AIM:



To study the effect of the two **mutations** (W29R, V139E) identified in **P1** of mild subisolates of the M-type PPV PS isolate on **virus pathogenicity**

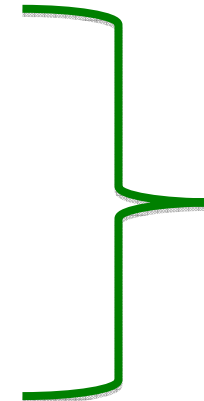


To further identify the **host specific pathogenicity determinants** laying at the **N terminal CP**

A) Host-specific effects of the two amino acid heterogenetities in the P1 protein

Site directed mutagenesis to introduce the point mutations (single and double) in a full length cDNA clone (cPSes)

- cPSes
- W29R
- V139E
- W29RV139E



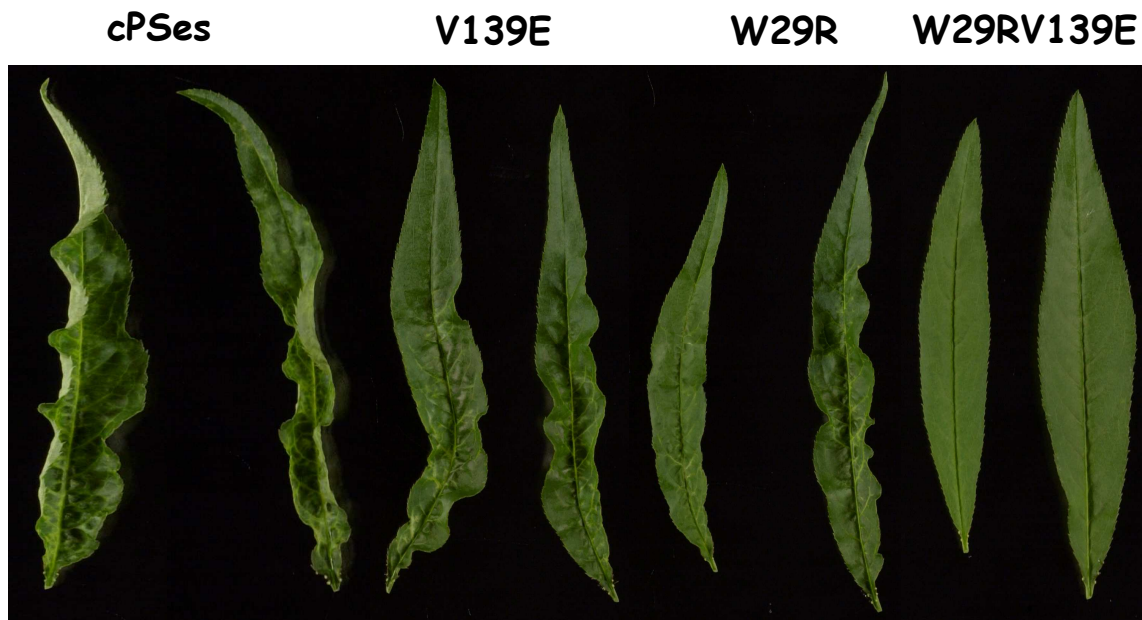
The generated mutants were used for inoculations of Prunus (GF305) and herbaceous plants

*Comparisons with the mild subisolate 1.3.1., which includes both the W29R and V139E mutations

Infectivity of PPV-PS mutants in peach GF305 seedlings

Biolistic inoculation

Virus		Symptoms
1.3.1.	-	-
cPSes	Strong vein clearing and leaf distortion	+++
V139E	Mild vein clearing and leaf distortion	++
W29R	Very mild vein clearing and leaf distortion	+
W29RV139E	-	-

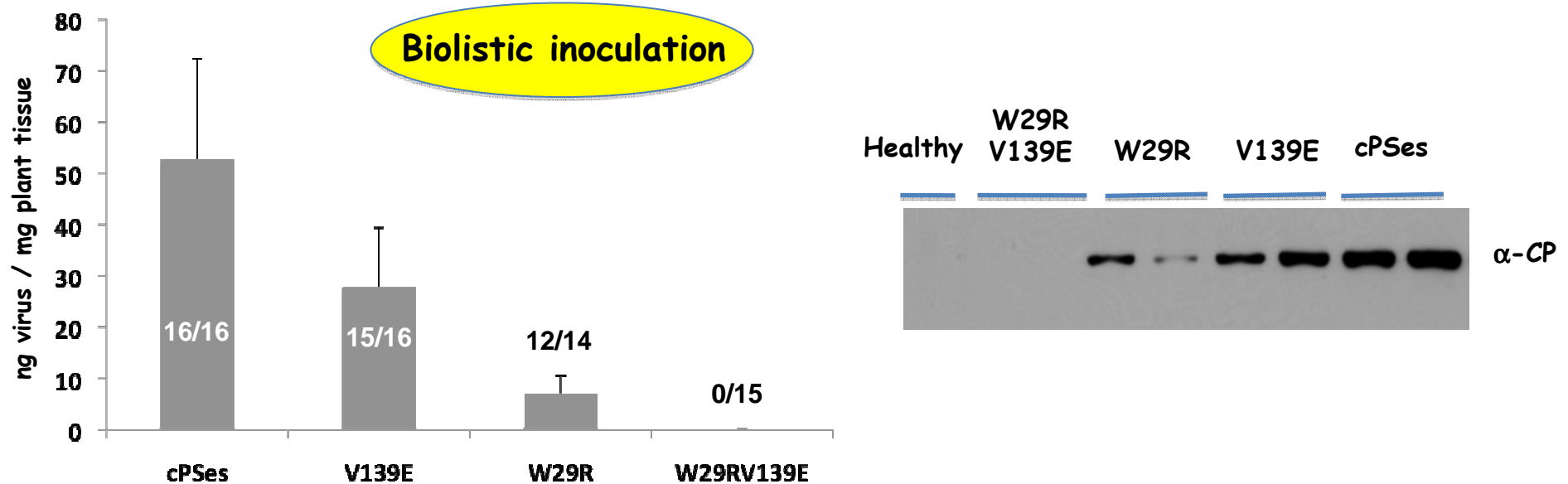


W29R mutant : mild symptoms and lower infectivity

V139E mutant : decreases but in lower levels the virus infectivity and symptomatology

W29RV139E mutant : cannot infect prunus

Infectivity of PPV-PS mutants in peach GF305 seedlings



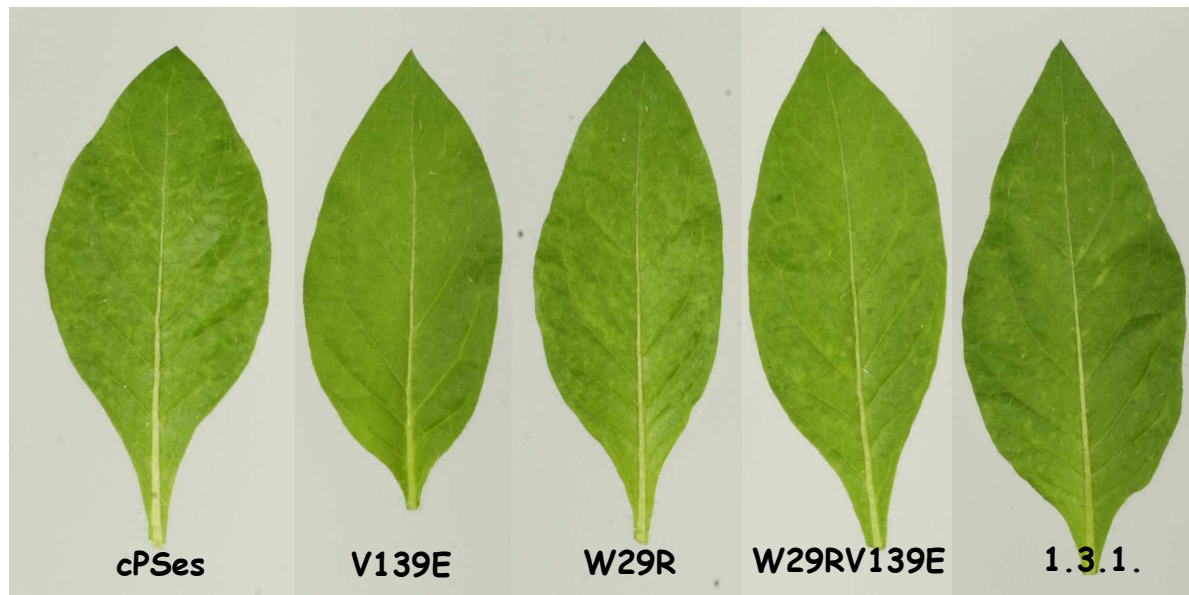
- The **W29R** mutant accumulates in lower levels than the wild type
- The **V139E** mutation also decreases but in lower levels the virus accumulation
- The double mutant **W29RV139E** cannot infect prunus

Infectivity of PPV-PS mutants in *N. clevelandii*

Biolistic inoculation

Manual inoculation

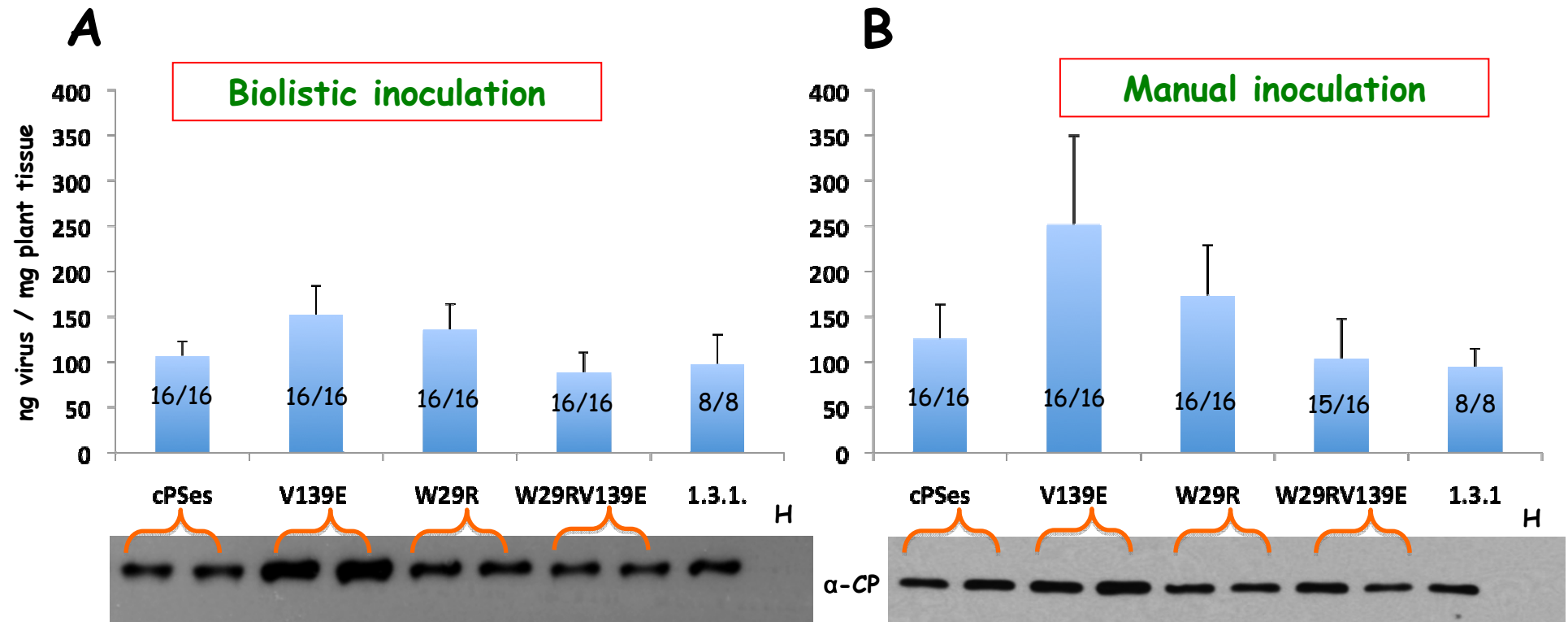
Virus	Symptoms
1.3.1	Very mild chlorotic mottling +
cPSes	Strong chlorotic mottling +++
V139E	Very mild chlorotic mottling +/-
W29R	Strong chlorotic mottling ++
W29RV139E	Mild chlorotic mottling +



✧ V139E mutant : symptoms attenuation in *N. clevelandii*

✧ W29R : low effect in symptoms

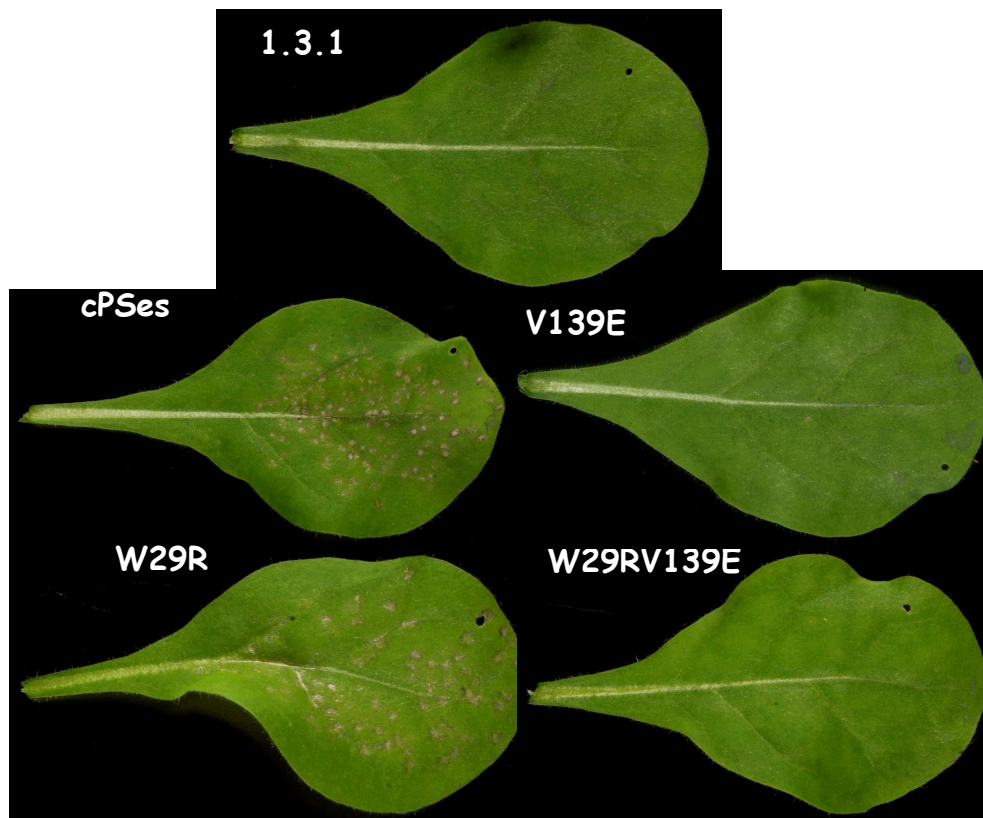
✧ W29RV139E : mild symptomatology similarly to the mild sub-isolate 1.3.1.



- ✧ The **V139E** mutant induces symptoms attenuation in *N. clevelandii* without any decrease on the virus accumulation
- ✧ The double mutant **W29RV139E** accumulates in low levels similarly to the mild sub-isolate 1.3.1.

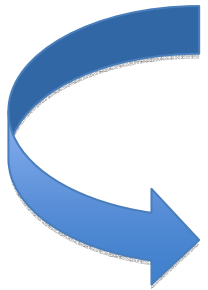
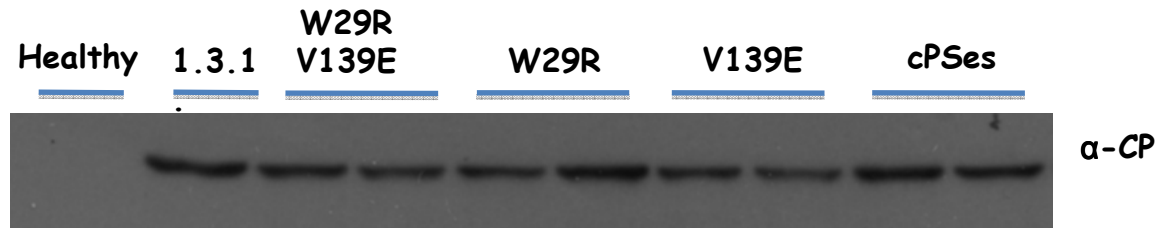
Infectivity of PPV-PS mutants in *N. occidentalis*

Virus	Lesiones
1.3.1.	-
cPSes	++
V139E	-
W29R	++
W29RV139E	-

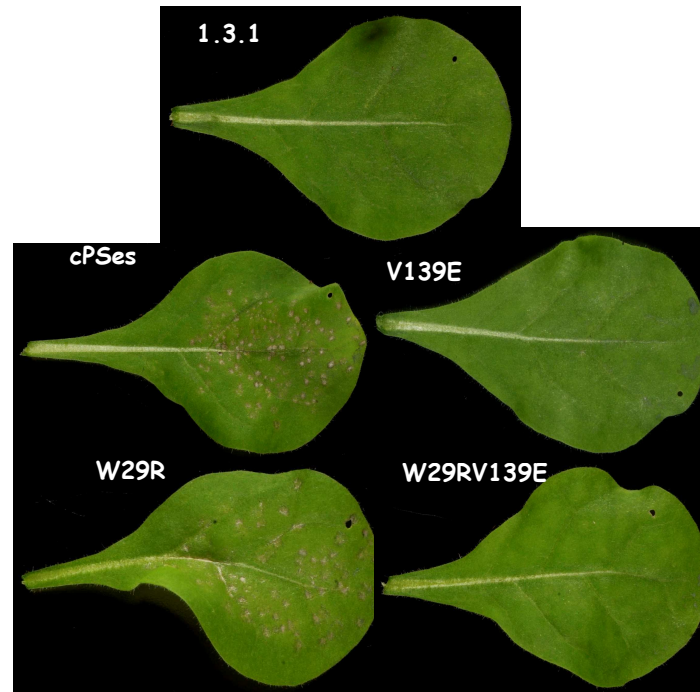


- ❑ The **V139E** and **W29RV139E** mutants don't produce necrotic lesions on the inoculated leaves of *N. occidentalis* similarly to the mild sub-isolate 1.3.1.
- ❑ In this host V139E has a strong effect in attenuation and W29R has not

Infectivity of PPV-PS mutants in *N. occidentalis*



All viruses accumulate similarly on the inoculated leaves

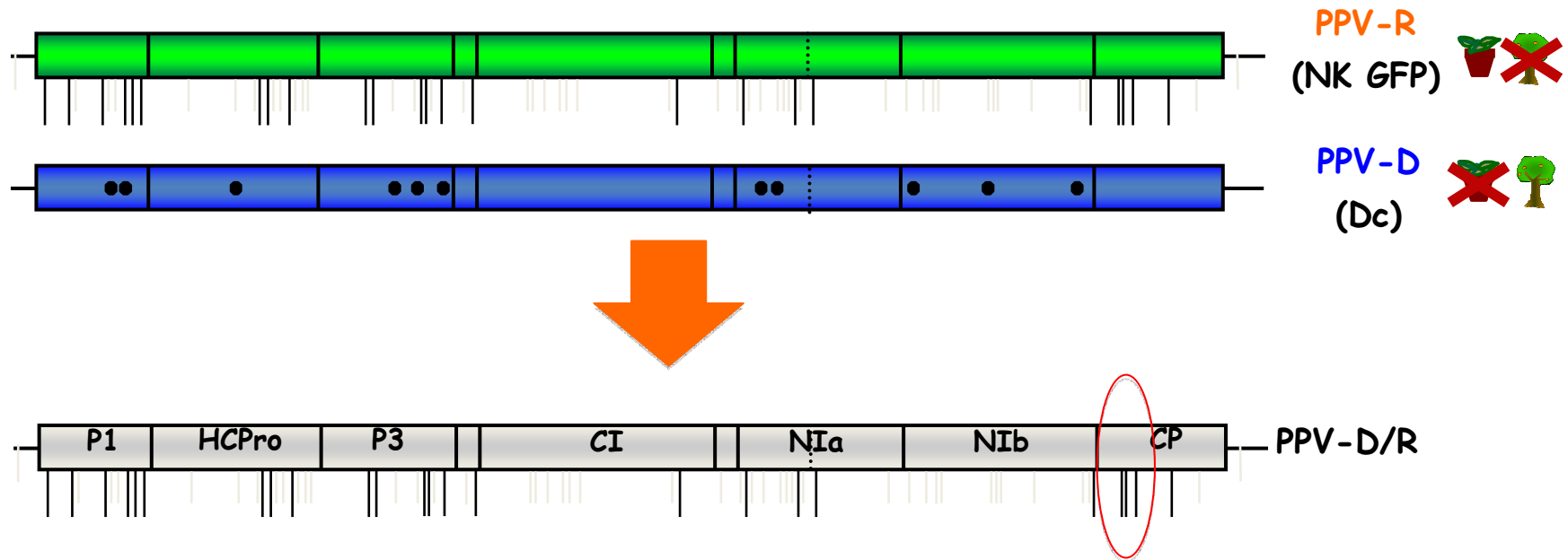


..... Conclusions

✓ The results highlight the relevance of the P1 protein in host adaptation by potyviruses.

✓ The difference in PPV pathogenicity in *Nicotiana* and *Prunus* based on the presence of V139E and W29R substitutions, supports the existence of species-specific interactions between P1 and host factors that influence host-specific virus infectivity.

B) Host-specific pathogenicity determinants at the N terminal CP



CP protein N-terminal region is highly variable and includes 3 out of the 4 amino acid changes between PPV-D and PPV-R

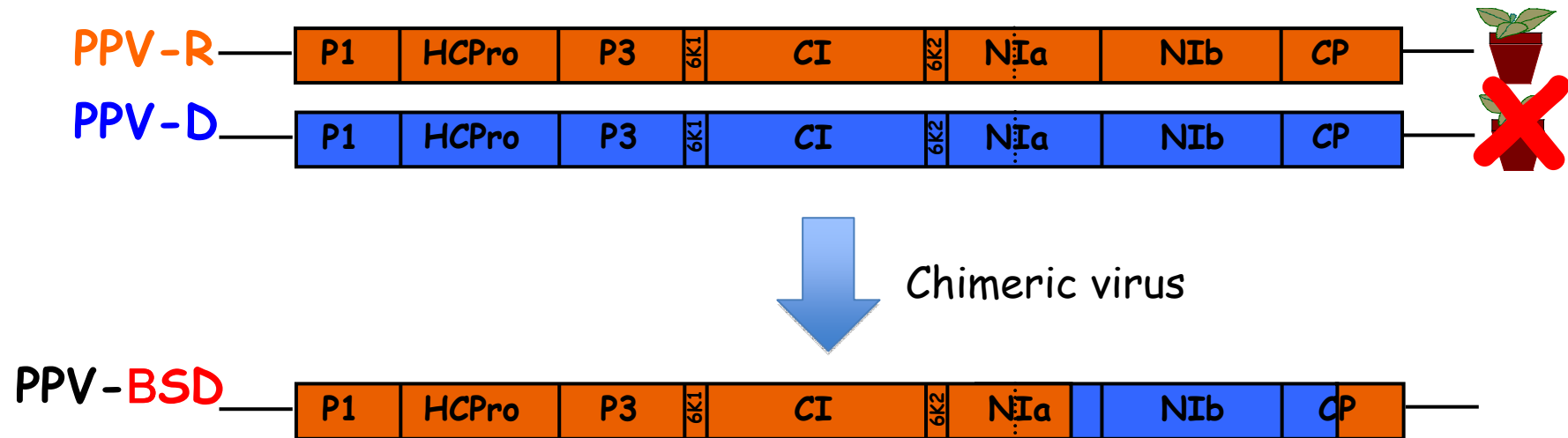
The change (S16I) lays in the region affected by the natural

NAT deletion

ADEREDEEEV DAGKPSVVTA PAATSPTLQP PPVIQPAPRT TASMLNPIFT PATTQPATKP VSQVSGPQLQ TFGTYGNEDA SPSNSNALVN TNRDRDVDAG
I.....P.....P.....

PPV-R
PPV-D

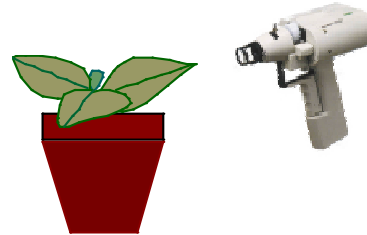
Use of a chimeric virus that includes the N terminal region of the CP of PPV-D



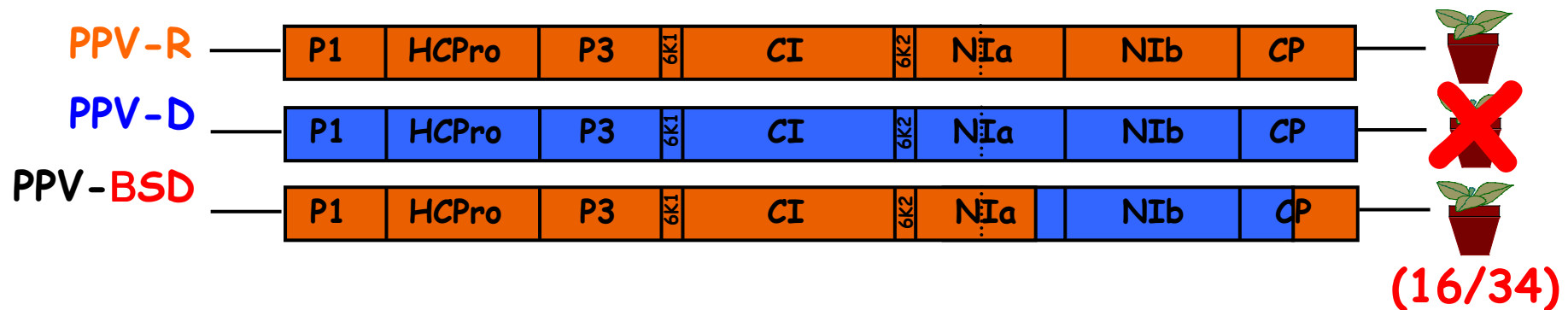
The construct was used in biolistic inoculations of herbaceous plants (*N. clevelandii* and *N. benthamiana*)

Results:

N. clevelandii



Systemic infectivity



- PPV-BSD is able to systemically infect *N. clevelandii* if the sequence in the N-terminal region of the CP is modified

Plant species	Chimeric virus	Selected mutation	No. of plants	Symptoms (21 dpi)	Virus accumulation (Western blot)
<i>N. clevelandii</i>	PPV- BSD	V18A	6	(-) - +++	(+/-) - (+++)
		I16S	3	(-) - (+)	(+/-) - (+++)
		I16V	2	(-) - +++	++
		I16T	3	(-) - (+)	(+) - (+++)
		D11G	1	-	+/-
		Δ(7-36)	1	-	+

- Five amino acid changes and 1 deletion, in a short region of 30 amino acids, were identified in the progeny of the infected plants

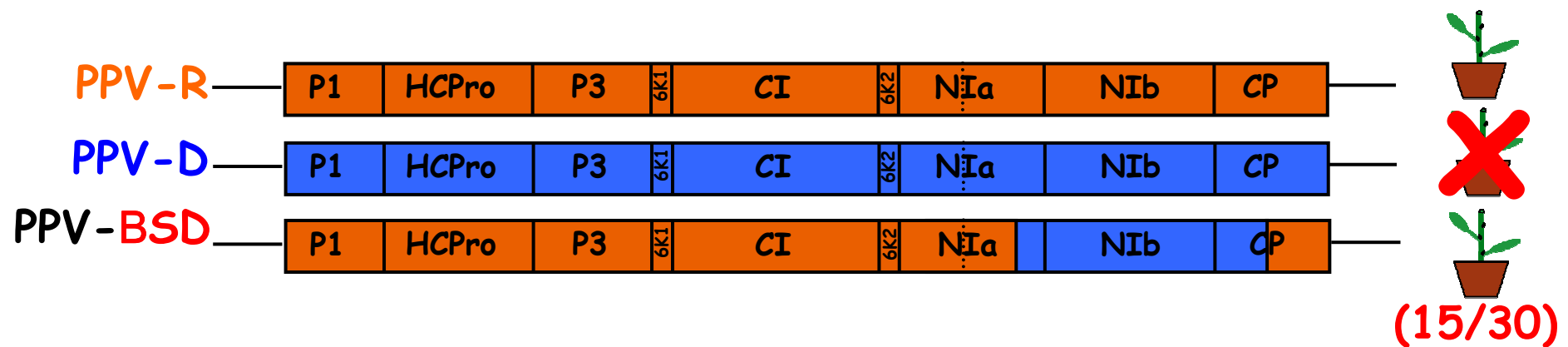


	7	11	16	18	36
PPV-R	ADEREDEEEVDAGKPSVVTAPAATSPI				
PPV-DI.....P.....				
PPV-BSD (6 PLANTS)I.A.....P.....				
PPV-BSD (3 PLANTS)S.....P.....				
PPV-BSD (2 PLANTS)V.....P.....				
PPV-BSD (3 PLANTS)T.....P.....				
PPV-BSD (1 PLANT)G.....I.....P.....				
PPV-BSD (1 PLANT)- - - - -.....P.....				

N. benthamiana



Systemic infectivity



- In most of the cases the **sequence** in the N-terminal region of the CP of PPV-**BSD** is **modified** in order to enable the systemic infection of *N. benthamiana* (but not in all)

Plant species	Chimeric virus	Selected mutation	No. of plants	Symptoms (30 dpi)	Virus accumulation
<i>N. benthamiana</i>	PPV-BSD	I16T	1	+++	+
		I16V	2	+++	++
		K14R	1	+/-	+++
		V18A	1	+	+++
		NO	10	(-) - (+)	(+) - (+++)

➤ Four amino acid changes were identified in the same region in the virus progeny in *N. benthamiana*

➤ Some of the virus progeny didn't include any mutations in the under study genomic region



	11 141618	
PPV-R	ADEREDEEEVDAGKPSVVTAPAATSPI	LQPPPVVIQPAPRTTASMLNPIFT
PPV-DI.....	P.....
PPV-BSD(2 PLANTS)V.....	P.....
PPV-BSD(1 PLANT)T.....	P.....
PPV-BSD(1 PLANT)R.I.....	P.....
PPV-BSD(1 PLANT)I.A.....	P.....
PPV-BSD(10 PLANTS)I.....	P.....

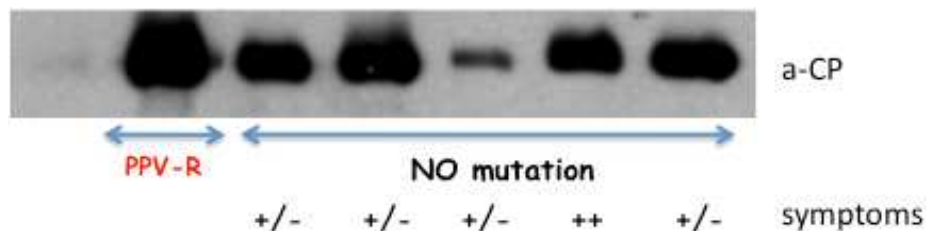
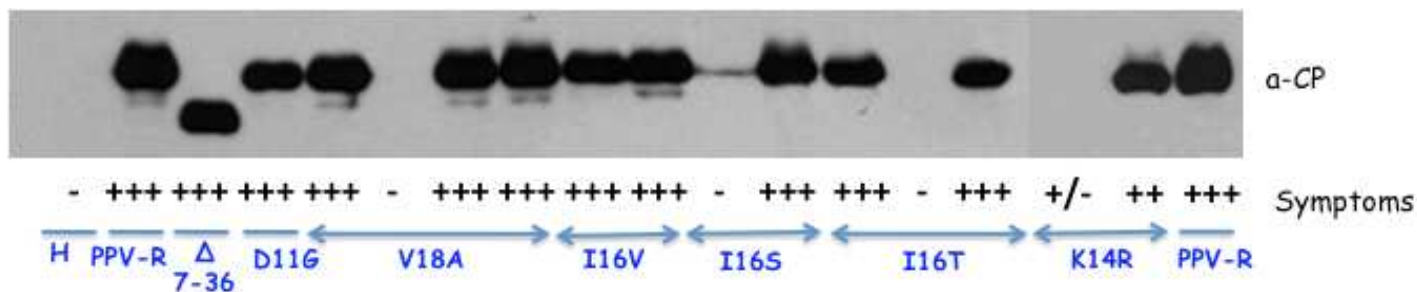
2nd passage to assess: a) the stability and b) the contribution to the virus fitness of the selected mutations

Mutation	Plant species	No. of plants selected
NO	<i>N. benthamiana</i>	4
K14R	<i>N. benthamiana</i>	1
I16T	<i>N. benthamiana</i>	1
	<i>N. clevelandii</i>	1
I16S	<i>N. clevelandii</i>	2
I16V	<i>N. benthamiana</i>	1
	<i>N. clevelandii</i>	1
V18A	<i>N. benthamiana</i>	1
	<i>N. clevelandii</i>	2
D11G	<i>N. clevelandii</i>	1
Δ7-36	<i>N. clevelandii</i>	1
PPV-R	<i>N. clevelandii</i>	Positive control

Inoculations of *N. benthamiana* and *N. clevelandii* with each mutant

N. benthamiana

- similar levels of accumulation and symptomatology with the wild type (PPV-R) were observed
- Lower pathogenicity of the K14R mutant

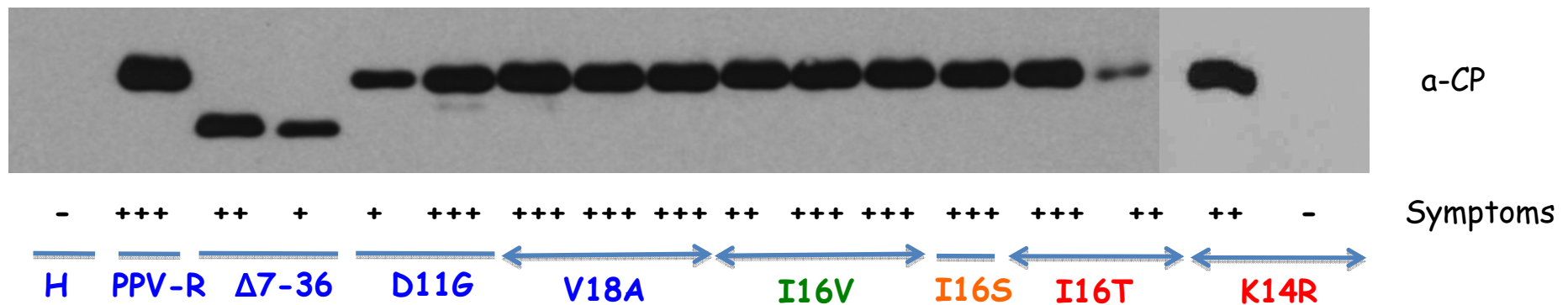


****** The virus progeny of the plants inoculated with the PPV-BSD showing **no mutations in the 1st infection**, showed symptoms and virus accumulation at a late point of the infection (36 dpi)

N. cleavelandii

✧ similar levels of accumulation with the wild type were observed

✧ Lower infectivity of the K14R mutant

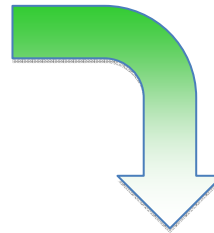


Sequence analysis

N. benthamiana

□ In all cases the amino acid changes were stable

□ In the case of one K14R inoculated plant a second mutation (T19P) was also introduced in the virus progeny

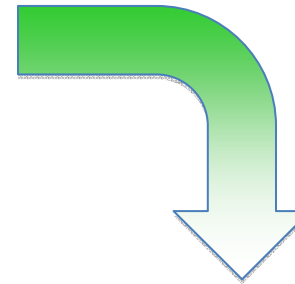


The virus progeny of the plants inoculated with the PPV-BSD showing no amino acid changes in the 1st infection, accumulated mutations after the passage

PPV-D	ADEREDEEEVDAGKPIVVTAPAATSPILQPPPVIIQPAPRTTAPMLNPIFT
PPV-RS.....S.....
D11G (3 plants)G.....
Δ7-36 (3 plants)- - - - -.....
I16S (3 plants)S.....
I16T (3 plants)T.....
I16V (3 plants)V.....
V18A (3 plants)A.....
K14R (1 plant)R.....P.....
K14R (2 plants)R.....

N. clevelandii

- ✓ The amino acid changes, except from K14R, were stable
- ✓ In the case of D11G mutant one more nucleotide change was introduced which led to amino acid change V18G

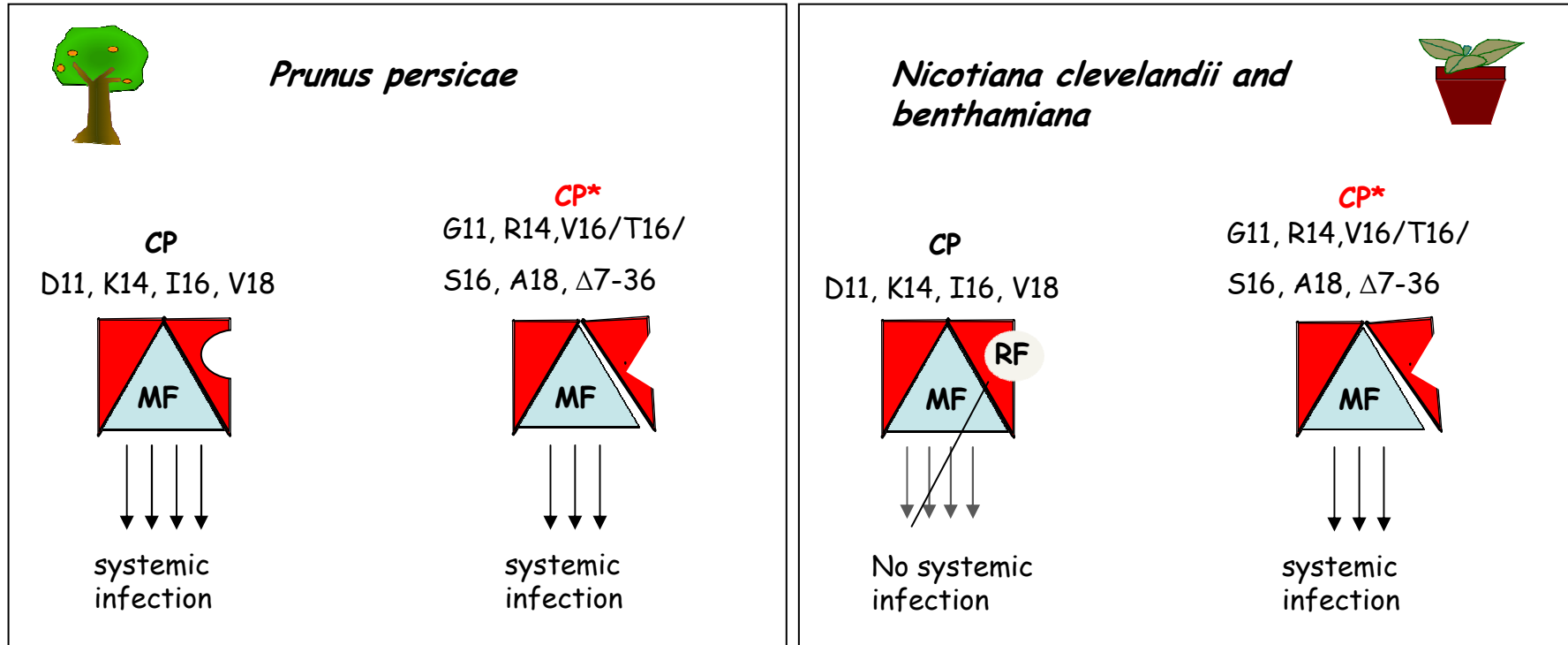


PPV-D	ADEREDEEEVDAGKPIVVTAPAATSPILQPPPVIQPA	RTTAPMLNPIFT
PPV-RS.....	S.....
D11G (3 plants)G.....G.....
Δ7-36 (3 plants)- - - - -
I16S (3 plants)S.....
I16T (3 plants)T.....
I16V (3 plants)V.....
V18A (3 plants)A.....
K14R (1 plant)G.....
K14R (1 plant)R.....P.....
	/G	

Natural variability in the N-terminal region of the CP

		Strains of PPV						
		D	M	C	Ea	W	Rec	T
Number of sequences		333	171	3	3	1	136	2
aa11		D (331) N (2)	D	D	D	D	D	G
aa14		K (296) R (2) N (1) Δ13-27 (3) Δ13-26 (1)	R (169) K(2)	K	R	R	K (135) R (1)	K
aa16		I (318) V (4) S (1) N (2) L (2) T (1) P (1) Δ13-27 (3) Δ13-26 (1)	T	T	L	A	T (135) P (1)	I
aa18		V (322) A (4) G(1) F(2) Δ13-27 (3) Δ13-26 (1)	V	T	T	L	V (135) I (1)	V

Model of host adaptation of the N-terminal sequence of the CP of PPV



Conclusions

- ◆ The CP N-terminal region of PPV has important determinants for the establishment of a systemic infection.
- ◆ The chimeric virus PPV-BSD is able to systemically infect plant species of the genus *Nicotiana* when various amino acid changes or deletions are incorporated in this small CP region.
- ◆ Different behaviour was observed between *N. benthamiana* and *N. clevelandii* plants - *N. benthamiana* seems to be more permissive to the infection by PPV-BSD (However the introduction of mutations is probably increasing the virus fitness and therefore are finally selected).
- ◆ The selected mutations were stable after one passage in *N. benthamiana* and *N. clevelandii* (except from K14R) plants - The introduction of a second mutation is in some cases beneficial.
- ◆ These results rise the hypothesis that the adaptation of PPV in these hosts involves avoidance of a resistance mechanism rather than improvement of a virus function.

Conclusions

The fact that the different amino acids found in the mutants that were selected under experimental conditions were also found in viruses isolated from natural infections indicates that:

the variability in the N-terminus of the CP may be relevant for the ability of the virus to adapt to different host species and different natural environments.

...Future work

Introduce the mutations in PPV-Dc and PPV-BSD and evaluate them in *Prunus* and herbaceous plants

Conclusions

✧ The viral pathogenicity and host adaptation of PPV are not strictly controlled by a specific part of the genome, rather they involve different genomic regions

Putative phytopathological implications of this work:

- Differentiation of virus isolates based on the sequence
- Prediction of the host range of a virus strain or the possibility for another plant species to be infected
- This knowledge could be also expanded to other members of the family *Potyviridae*