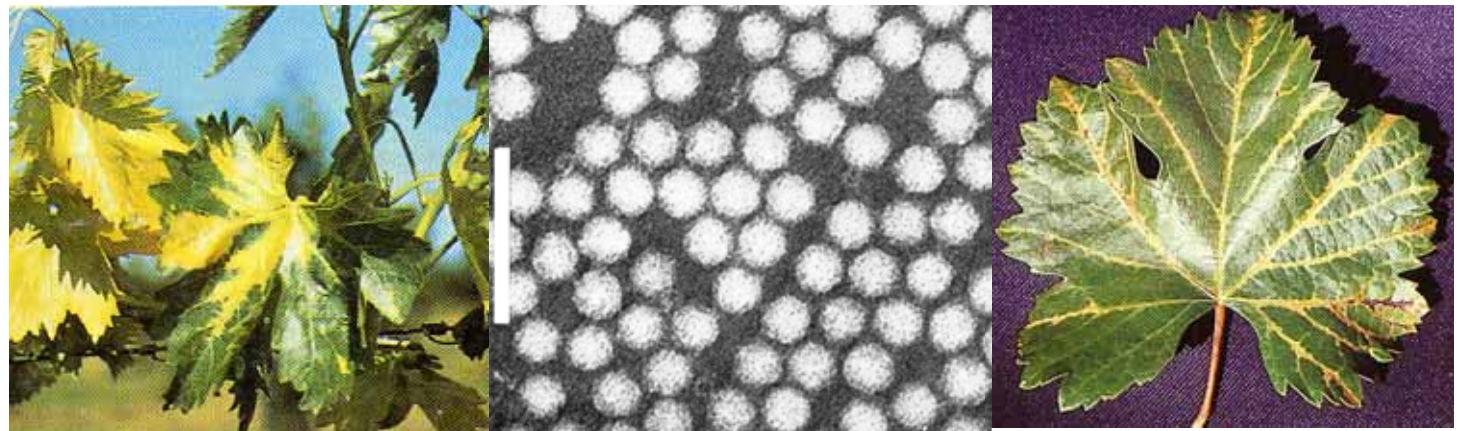
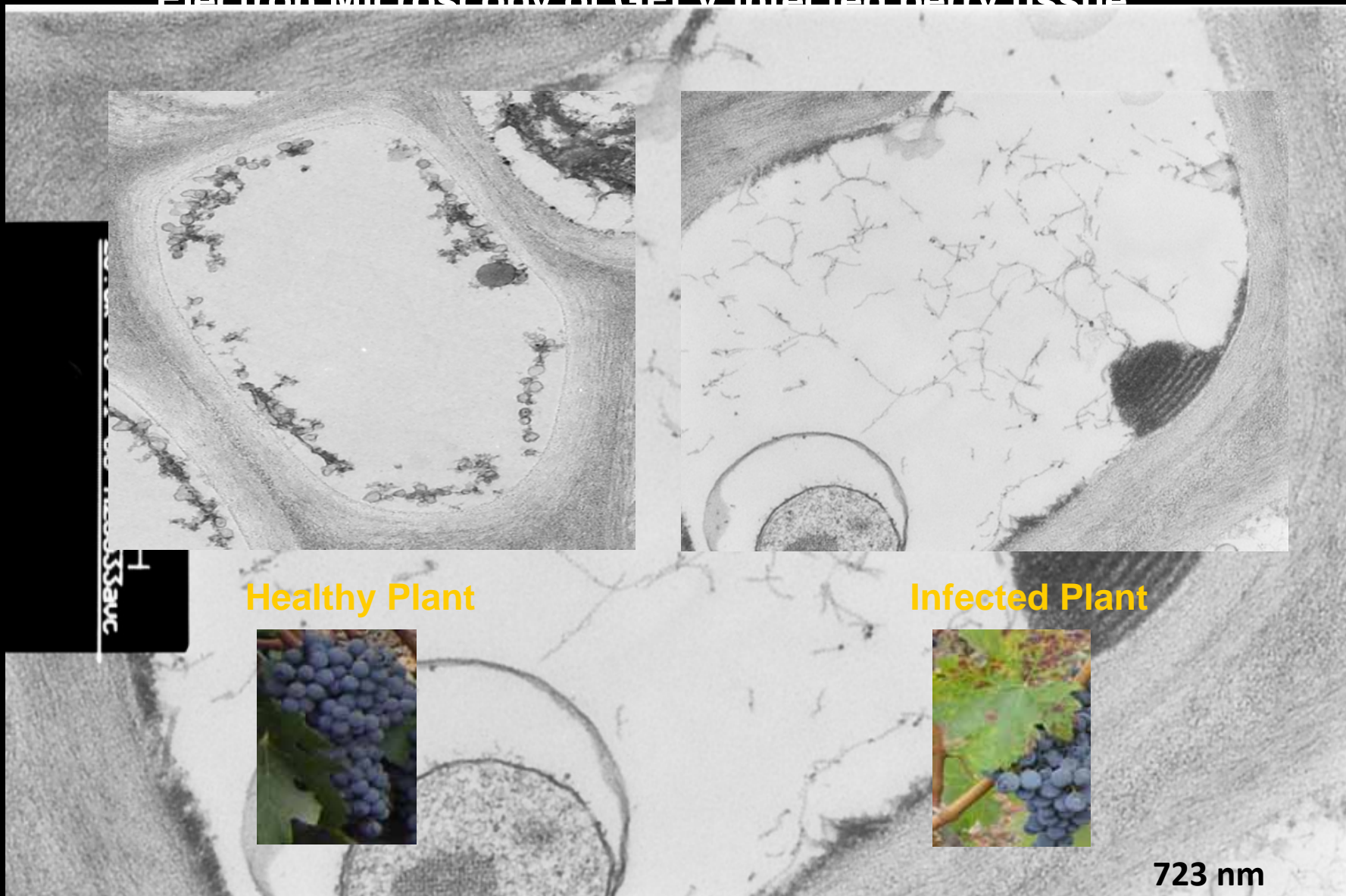




## Gene Silencing as Strategy to Induce Grapevine Fan Leaf Virus (GFLV) Resistance in Grapevine Rootstocks



# Electron Microscopy of GEL V infected berry tissue



Healthy Plant

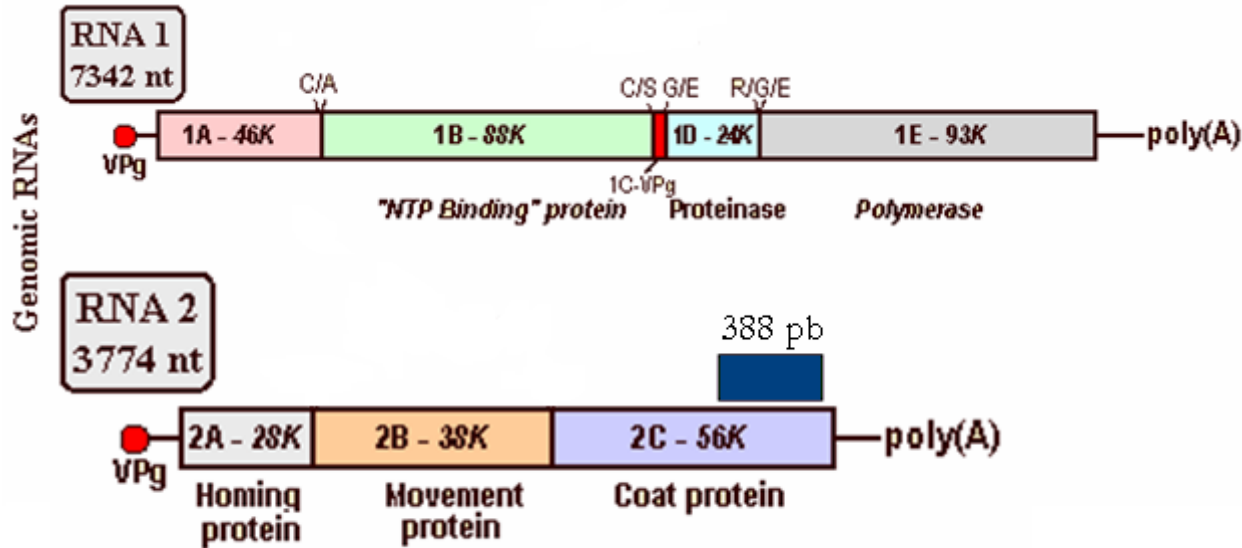
Infected Plant

723 nm

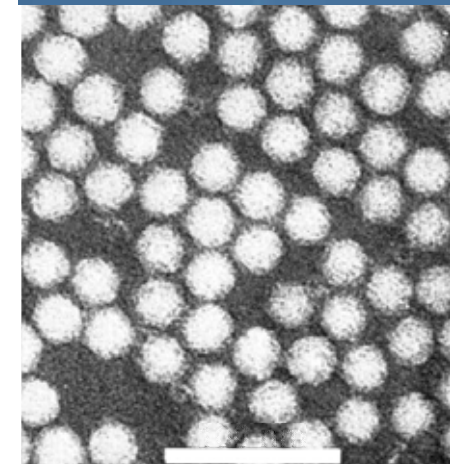
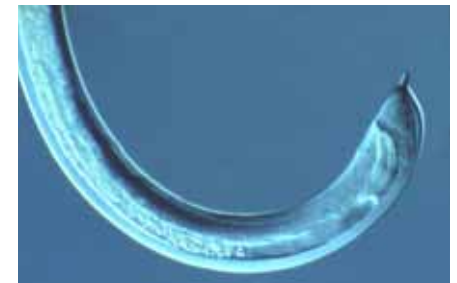
# GFLV Grapevine Fanleaf Virus



Genomics in Grapes

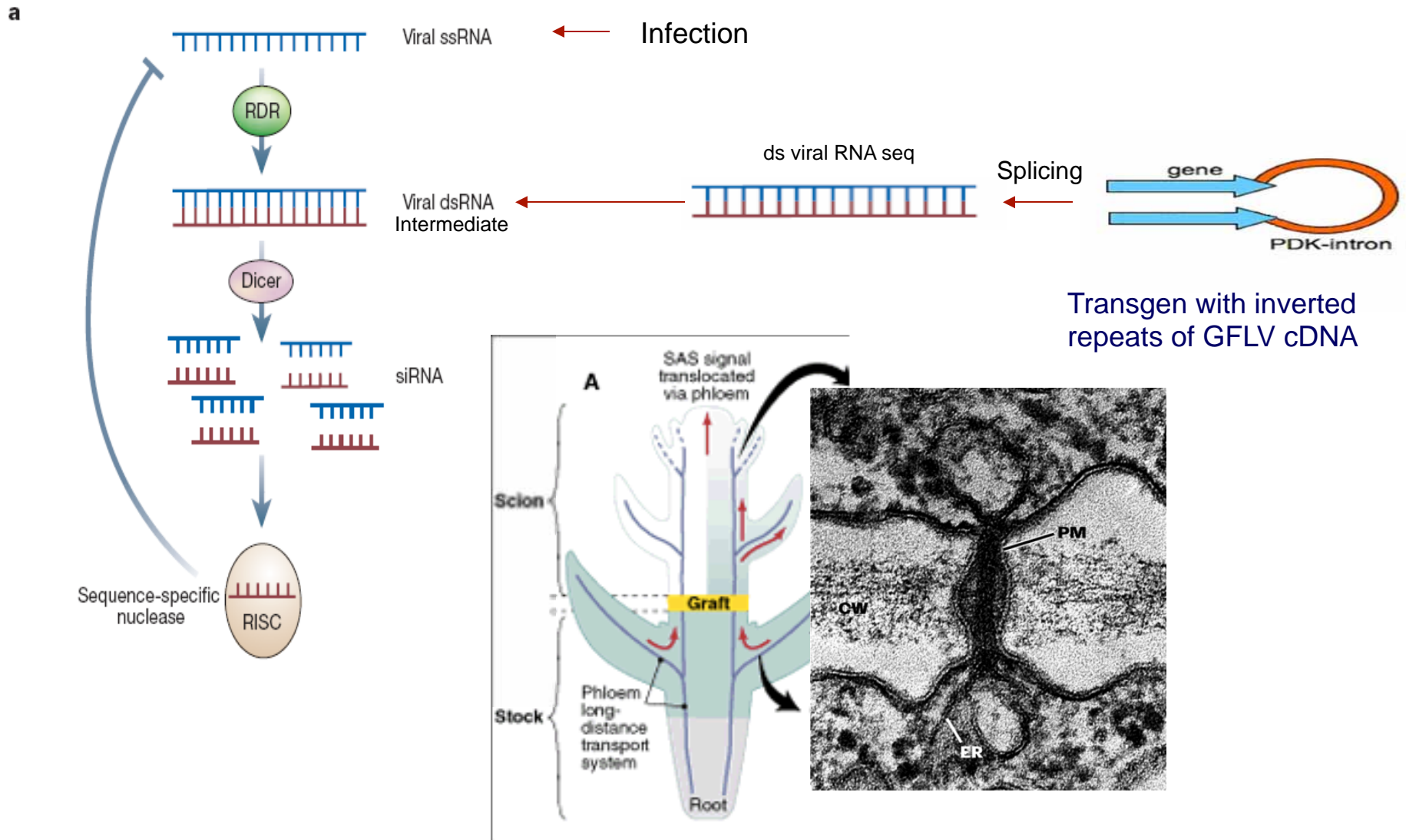


- It is transmitted by Nematode (*Xiphinema*) and cuttings & grafting
- Member to Nepovirus group
- Bipartite genome ssRNA
- Icosaedric particle of 30 Nm in diameter
- We selected a 388bp RNA sequence from CP coding region

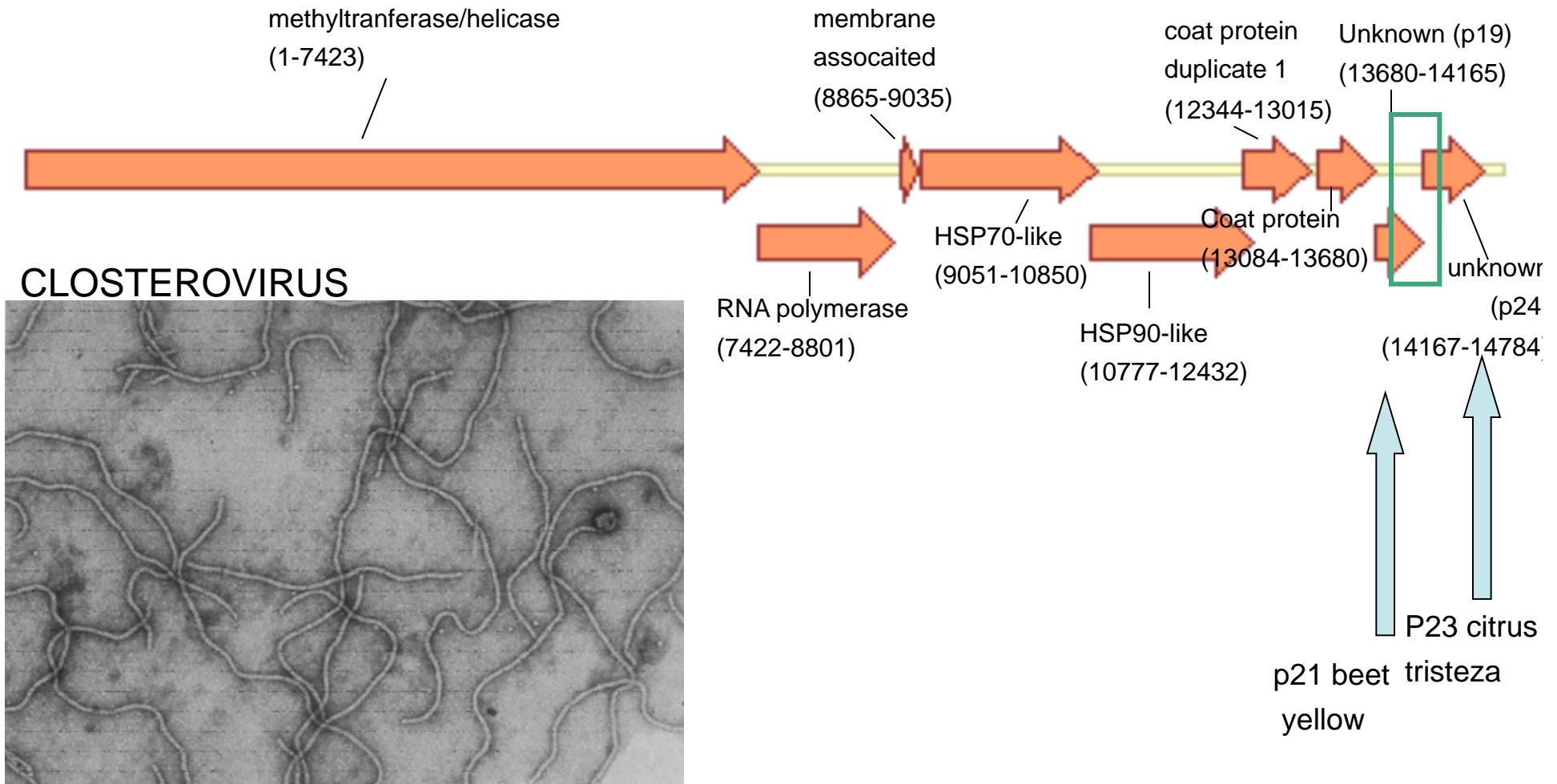




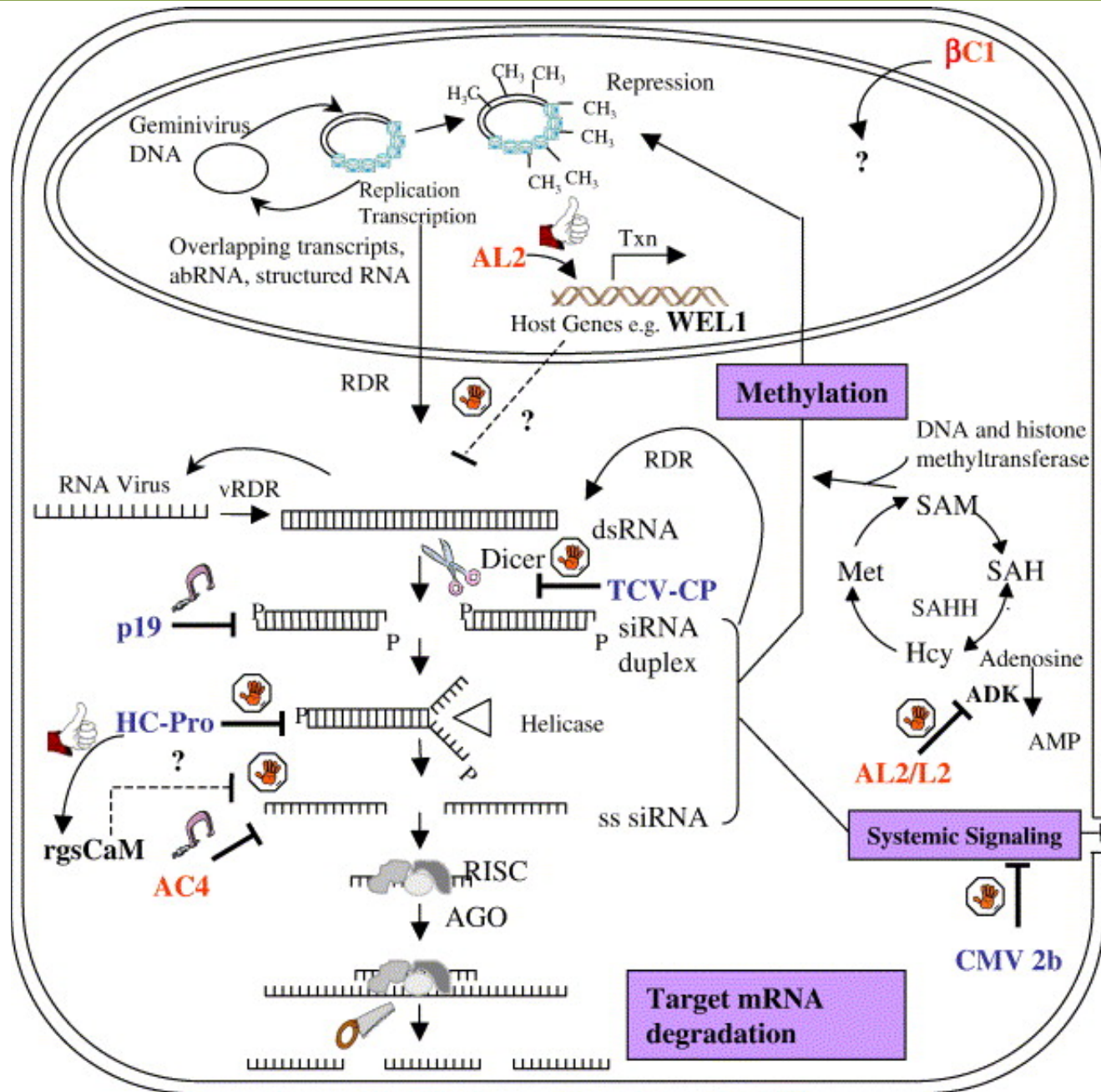
# PTGS MACHINERY TO GFLV RESISTANCE IN ROOTSTOCK GRAPES



# Grapevine Leafroll associated virus 2 (GLRaV2) 15000 bp



# Contra-defensa viral





Portainjertos de vides resistentes a nemátodos y filoxera utilizados por la industria frutícola



110 Richter  
(*V. berlandieri* x *V. rupestris*)



Ramsey (Salt Creek)  
(*V. champini*)



Freedom  
(*V. champini* x 1613)



Harmony  
(*V. champini* x 1613)



Rupestris du Lot  
(*V. rupestris*)

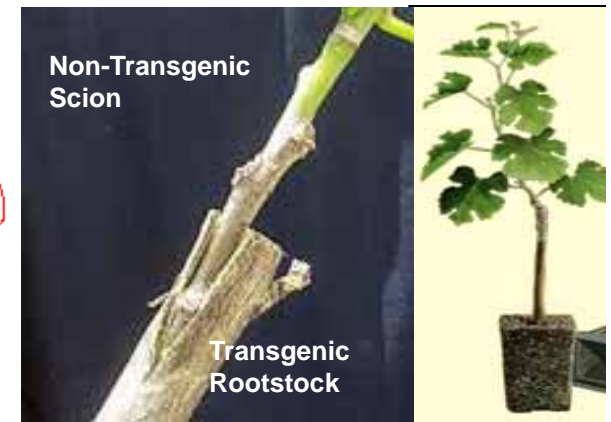
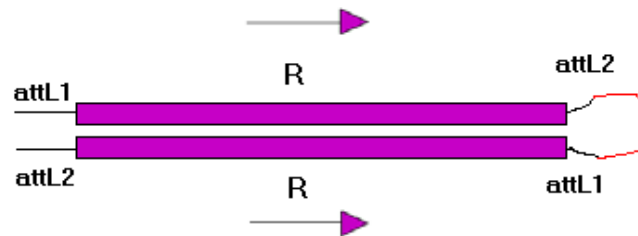
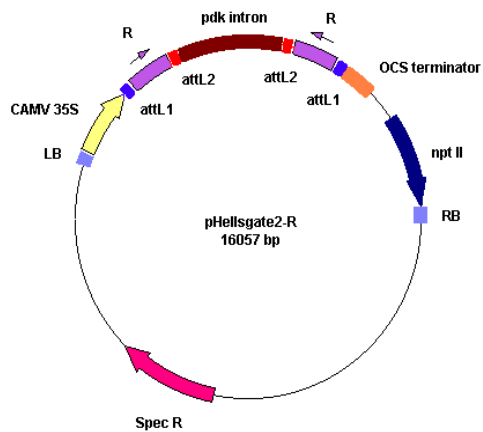


1616 Couderc  
(*V. longii* x *V. riparia*)

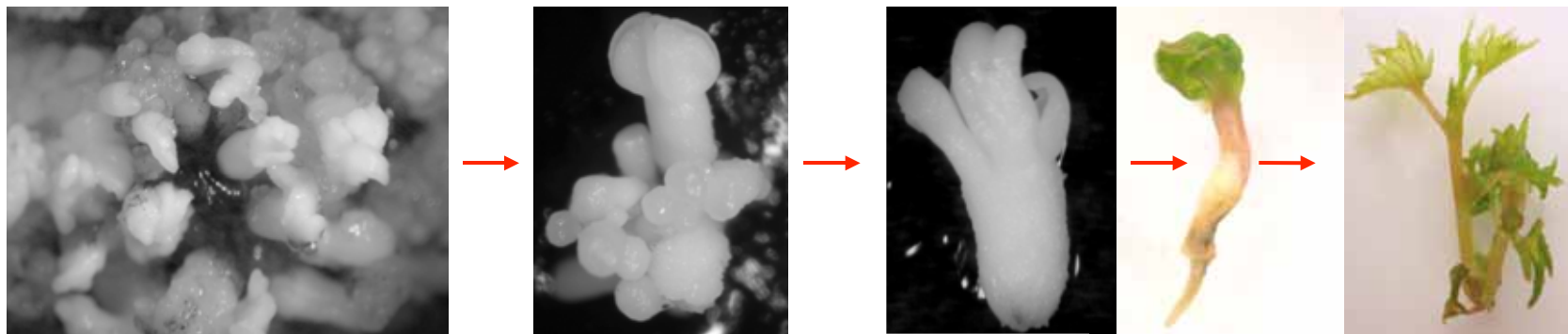
# Rootstock Transformation for GFLV Resistance



- Construction for grape GFLV silencing



- Rootstock Transformation for GFLV Resistance





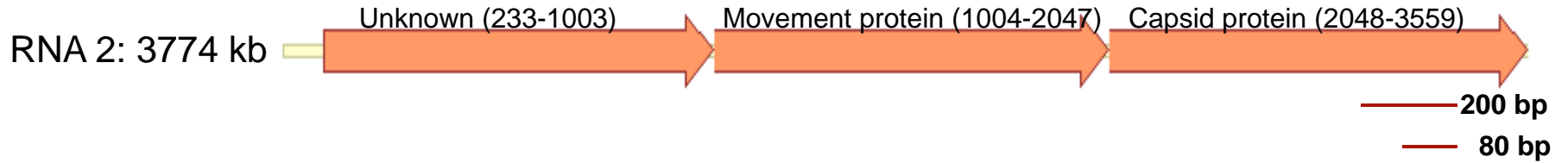
# Transgenic Rootstock lines Generated and Used in Grafting Experiments

Kanamycin Resistant Lines	PCR + Lines	RT-PCR + Lines Selected	Lines in Grafting Assay	Lines under evaluation
86	63	26	6	37

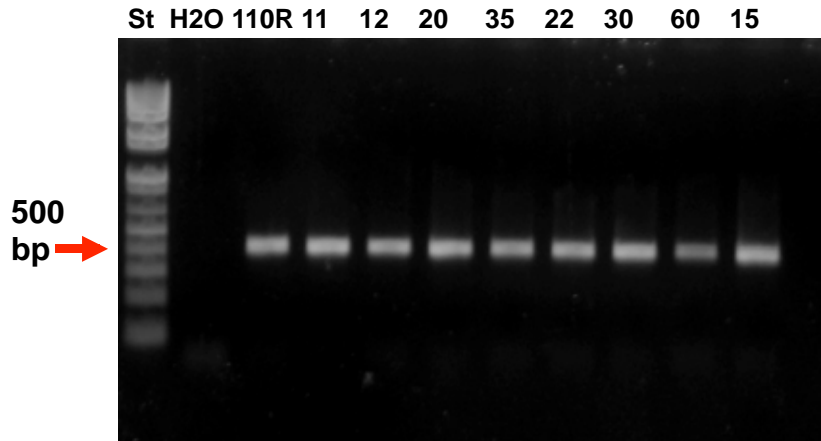
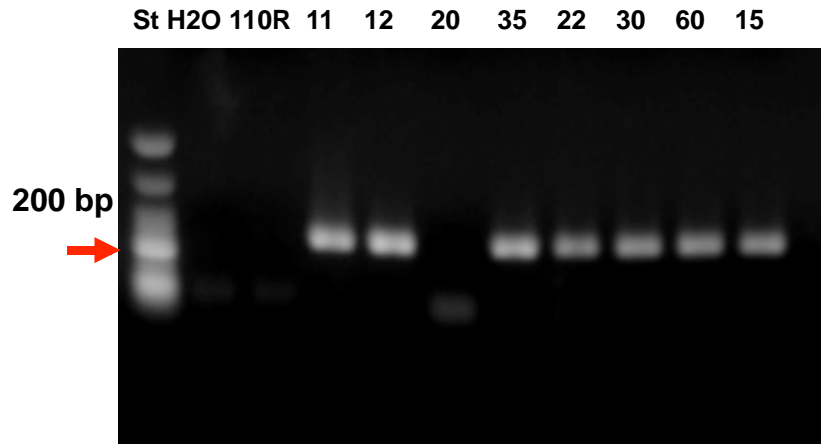
<i>Lines 110R GFLV</i>	<i>Clones Copy</i>
12	5
35	5
22	5
30	5
60	5
15	5



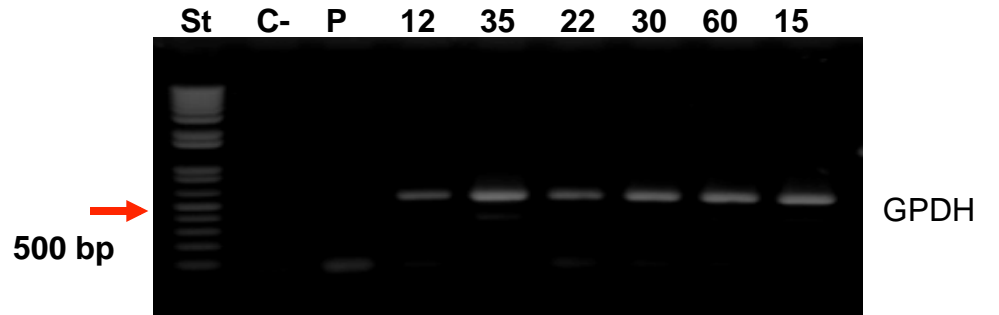
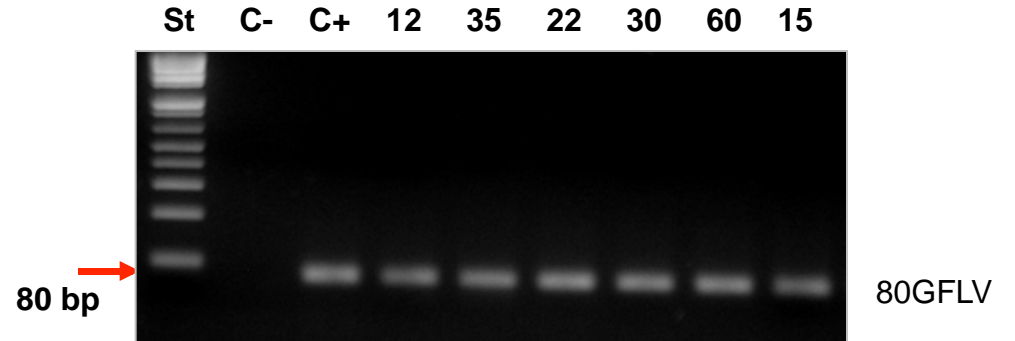
# Molecular Characterization of GFLV Transgenic Lines



## Transgen Integration (PCR)



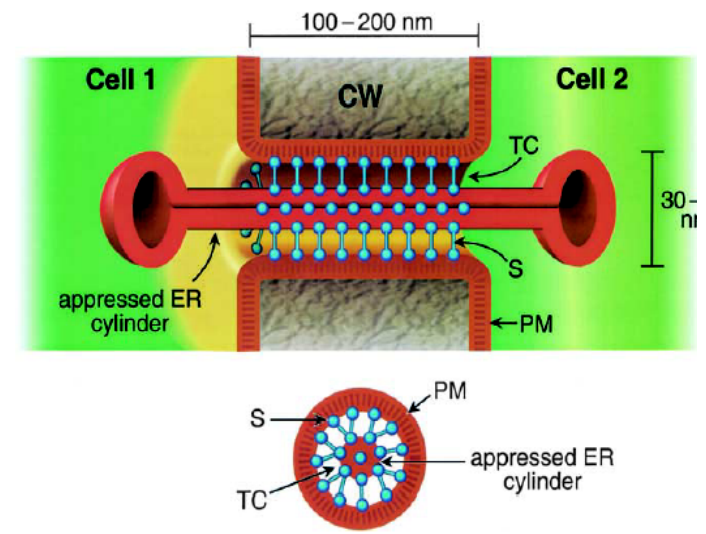
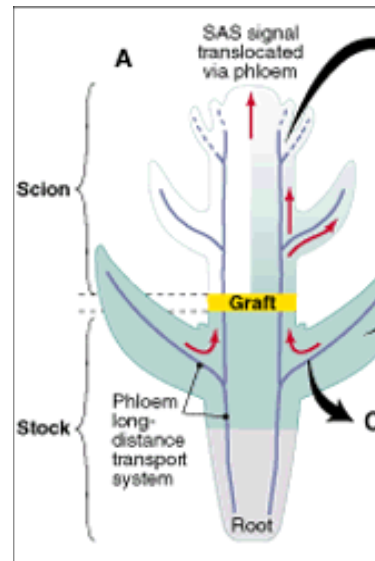
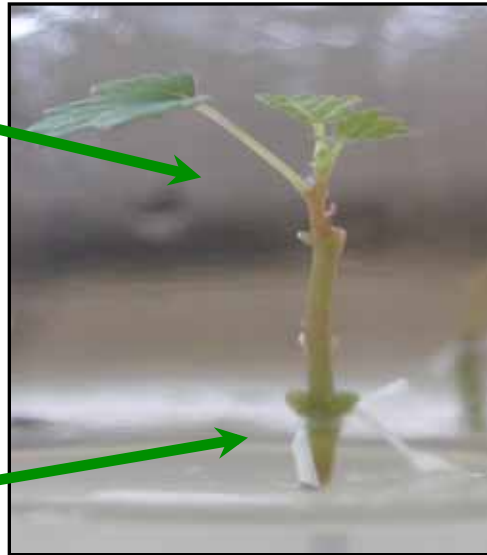
## Transgen Expression (RT-PCR)



# Evaluation of GFLV Resistance in Grapes by Grafting

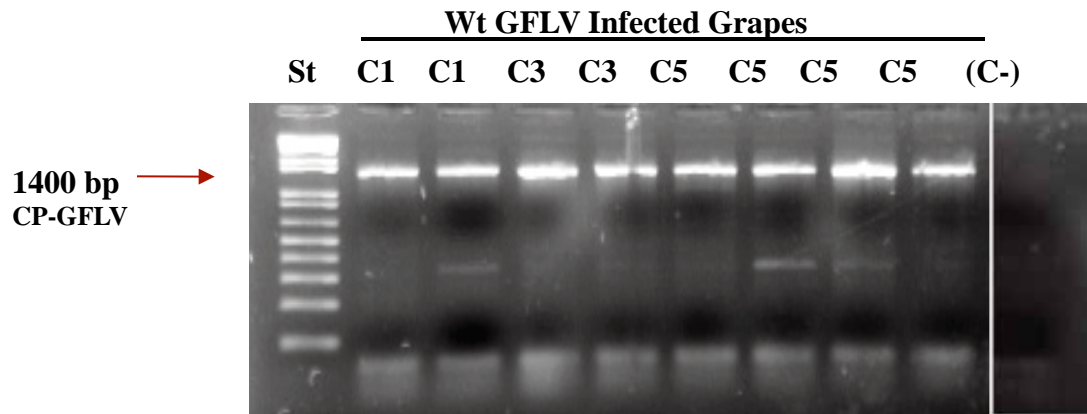
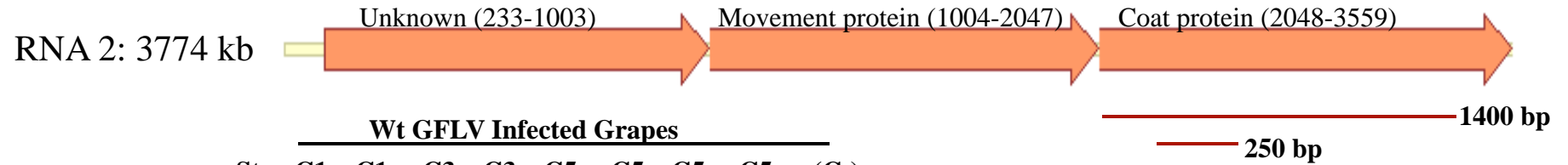
Infected GFLV Scion

Transgenic GFLV  
110R Rootstock Line

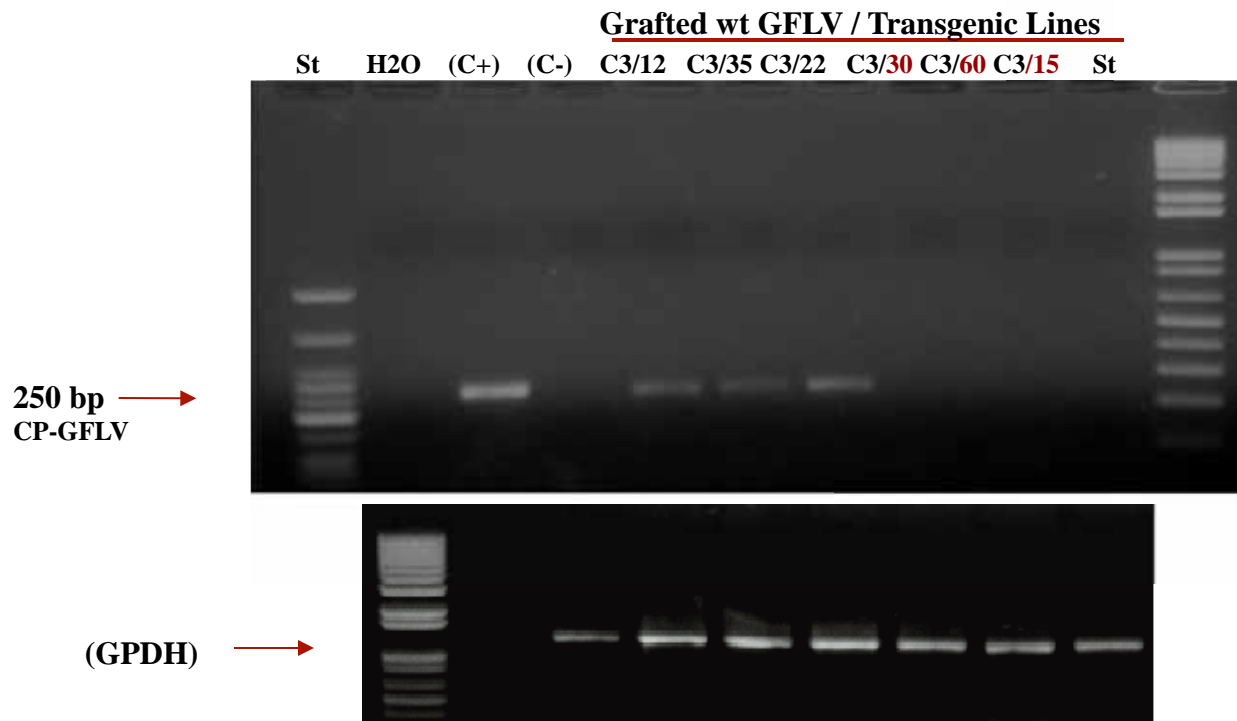




# Preliminary Evaluation of PTGS for GFLV in Grafting



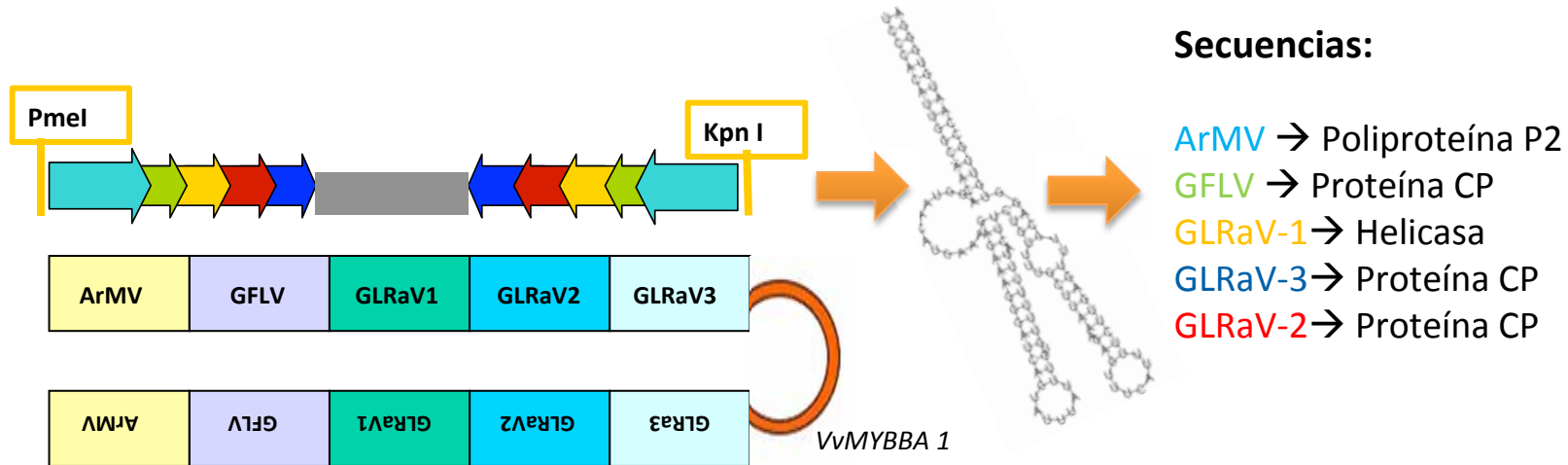
\*Absence of GFLV CP-RNA in infected grapes grafting 30 dpg



Lines 110R GFLV	GFLV Detection in Wt
12	5/5
35	4/5
22	4/5
30	0/5
60	0/5
15	0/5

Patente No. 201102884  
Fecha Solicitud: 16/11/2011

# Inducción de Silenciamiento a Múltiples Virus



```
GGTACCAATTGTGTGGTGATAAAGCTACTGGCATGGATGGTGCATCCCCTGTTTGTGCTTTGCCAAAATATGGGATGTGAAACTGAACTGTACATAGGTAATTTTGTGCTGGTCCAAATCCTAATTCAGCA  
TTATCTTTGTACAGTCGATGGCTTGCCATTAAGTTGGATAAAGGCTAGGAGCATGAAGATGCTCCGAATTTTATGCAAGCCAAGAGGGAATTTGAGTTTTATGGTAGAAGTTGTTTCACTTCTCACGTT  
TTTGCCATACAAAACTAGAGGGAACACAGAGTTGTTGGTTGGGAATTTTGCAGGAGCAAACCAACTCGTTTTTCCCTTTATAGTCGCTGGATGGCAATTAATGGATCAAGCAAAGAGTA  
TTAAAGTACTCCGCTTTTGTGCAAGCCCCGTCAGGCTTAGCTTTTATGGTAGAACAGACCCAAGTGATAGCTCTAGTCATGCAGAACGACGGATCTTACGTAGTGCTGGTTGGTTGCGAAGTG  
CATAATCGTCACCATAATTTTAAATGCTTGCGTTGTCGACGGTTAGCCTCAACAATGAGCGGAGATGTGTTCTGGACCGTTAAGATTCAATTTGAGGGAAATCGATGTTACCTACAGTTTATTG  
AGAATATTGTTACGATGGCGTCACTTATACCTAAGTATGTCGGAAGTTTCCGAACTTTGCGCACACATAGTCGAAATTTGCAATGCAGATAGCGTTTACTCTATGAAGCGCTCAAAGTGAAT  
CTCGGTGGTTGGGATTATCGTTCTCGACTTTCGTAGAGGAGACGCAAAAGGAACTGGAACAGACGCCATTCGGTTGGCAGCTATACCATGGAGTATTGTTTGAAGCAGGTTACTAACTGAACTGAAT  
CTATAATCGATTTCTTTTGCAGAAAGAAATCGTTAAGATGAGGGTTATAGTGCTCCTTATGAAGCTGAAGACATTCTGAAAGATCGACTGACATGTTACGAAACATAGACAGTGGGGTCTTGA  
GCACTAAAGAATGTATCAAGGCATTCTCGACGATAACGCGAGACCTACATTGTGCGAAGGCTTGGATCCGACGTTGCGAAAGTTAAGTATATTTGTCTGGGGAGAATCGAGGATTGGGGTGCGT  
AGATTCCTTGAAGCTAGTTTGCCTAGGTAGACAAGGAGGTGGAACGTAATACTACGCACTTACTAATCTCATCTCTGGTTAAAGCATCATGGACCTATCGTTTATTATTGTGCAGATCCTTTCCGCCTT  
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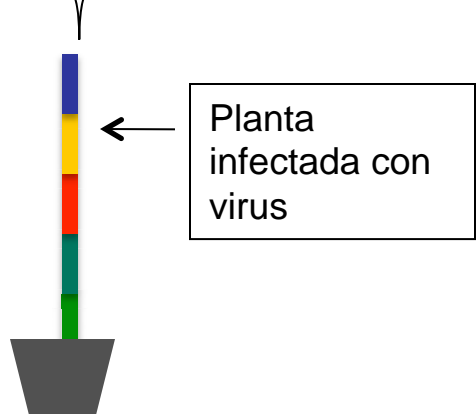
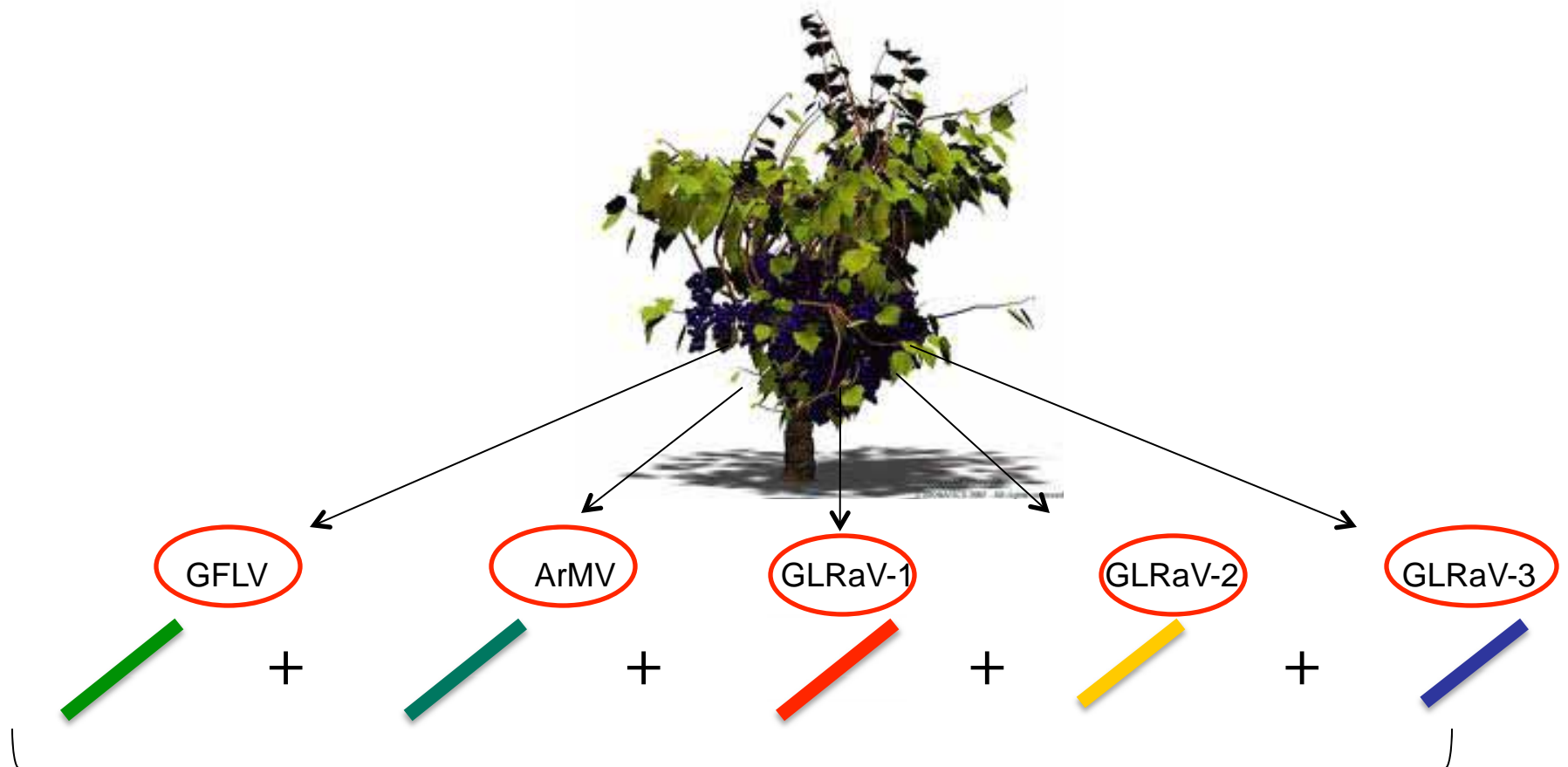
En GRIS se muestra la secuencia del intrón 1 de VvMYBA1 87 pb



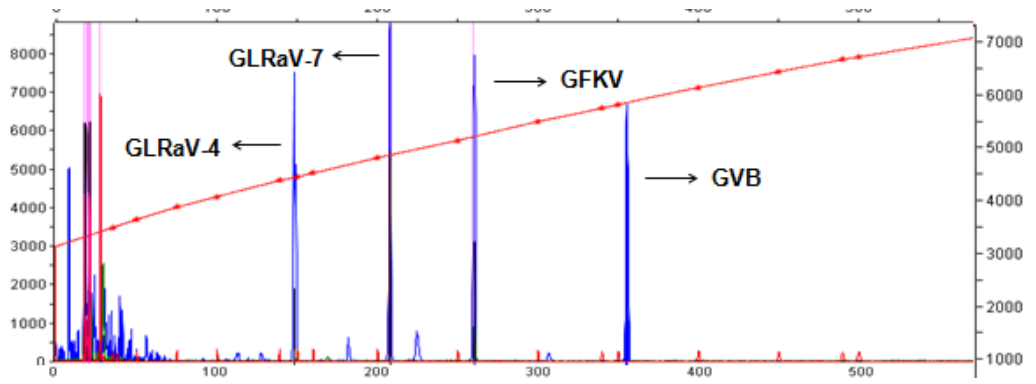
PM L1 L2 L3 L4 L5 L6 110R + L7 -

777 pb





Evaluación:  
 RT-PCR  
 Sistema detección múltiple por PCR y electroforesis capilar





Gracias por su Atención

