

Engineering the *Prunus* translation initiation factors in stone fruit trees for resistance to *Plum pox virus*



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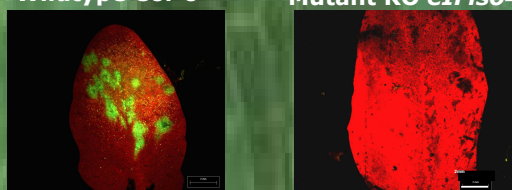
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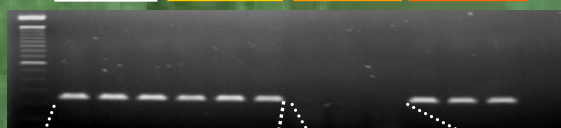
To infect plant tissues, the *Plum pox virus* (PPV) is recruiting host proteins such as the translation initiation factors, eIF4E and eIF4G (ref1)(ref2). In order to confirm the implication of these host factors in the development of sharka disease in stone fruit trees (*Prunus* species), orthologues were identified in peach and processed for silencing by genetic transformation via *Agrobacterium tumefaciens*.

1) Functional copies of the *eIFiso4E* and *eIFiso4G1* genes are indispensable for PPV infection in *Arabidopsis thaliana*

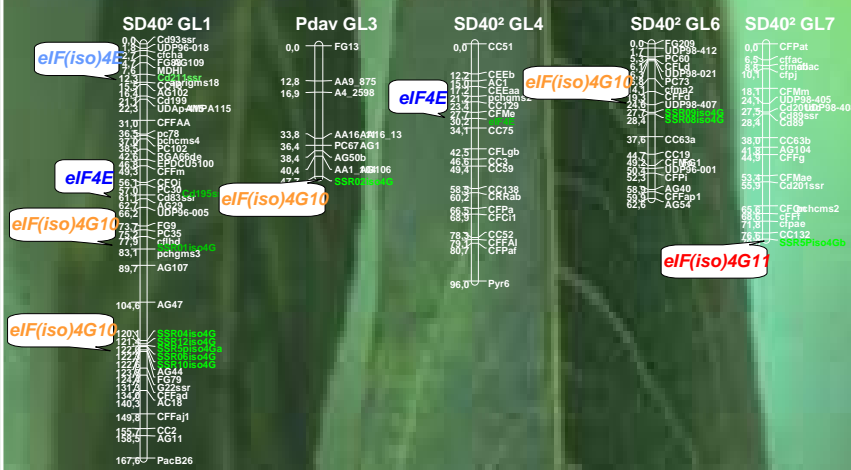
Inoculation with pBINPPVnKGFP of Wildtype Col-0 Mutant KO *eIFiso4E*



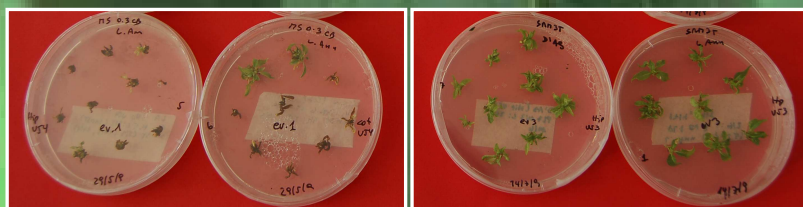
WT 4G iso4G1 iso4G2



2) Several *eIF4E* and *eIF4G* copies and isoforms in the Peach genome (ref3)



4) European and Japanese plum transformation with *Agrobacterium* (ref4)



eIFiso4E-hp-RNAi plantlets

4E-hp-RNAi plantlets

4 lines

36 lines



Regenerated plants verified by PCR, under molecular characterisation (level of gene expression etc...)

Resistance to sharka challenged by micrografting onto PPV-infected *P. insititia* rootstocks

References
Ref1: Decroocq et al., 2006. Molecular Plant-Microbe Interactions 19: 541-549
Ref2: Nicaise et al., 2007. FEBS Letters 581(5): 1041-1046
Ref3: Marandel et al., 2009. Molecular Plant Pathology 10(3): 347-360
Ref4: Urtubia et al., 2008. Plant Cell Report 27: 1333-1340.

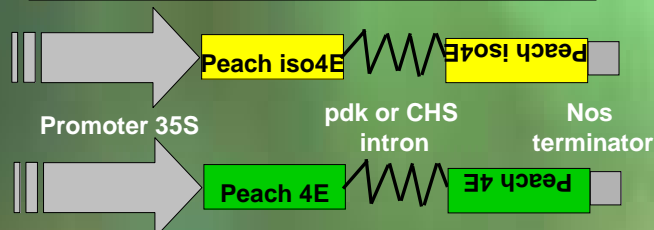


3) Hairpin-based copy specific RNAi constructs in peach

Alignment between 'GF305' peach *eIFiso4E* and *eIF4E* genes

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eIFiso4E  --TGGCGACAGAGGTAGCAGCAGCAGCAGTTC--GACGACAGTATGAGCGCTGAGGAGAA
eIF4E      GACCGAGACGACCCCTAAACCAATGGAAGAGATGAGCCCGA--AGAAG--GAGAGA
          ****
eIFiso4E  TACTGGGTTA-GAGGCGCGCGCGCGCGAGGC-----AAAGATACAGCGAGCAGTGGCGC
eIF4E      TCCTGGGCGACGAGGAATCGGCG-TGAGG-CATCGAAGGAATAGCGCCACAGT--G
          ****
eIFiso4E  CCACAAGCTGGAGGAGGAGTGGACCTTTGGTTCGATAACC-----AATCC--AA
eIF4E      TCATGCTCTGGAGCAGCTCATGAGCTTCTGCTTCGATACGCCCGCAGCAAGTCCGCTAA
          **
eIFiso4E  GCCCAAGCAGAGGTGCTGCTTGGGGTTCCTCTCTCGCAAGGCTACACCTTCGAAACGCT
eIF4E      GACTTAAGCAAGAGGAT--TGGGGGAGTTCATCGCCGAGTCAATACCTTCAATACCGT
          ****
eIFiso4E  TCAGAATTCGTGTCTGTATGATCAGGTATTCAGCCCAAGCAAGTTCCACCAATGCG
eIF4E      CGAGGAGTCTCGGAGCATATCAATATATATGAGCAATGCAAGCAATGCTGCTGCTGAG
          ****
eIFiso4E  AGATTTCACCTTGTTCAGGGCTGGGGTGAACCAAAATGGGAGGATCCTGAGTGTGCTAA
eIF4E      GAGCTTCAGATGTTTCAATATATAAATTGAGCCAAAGTGGGAGGATCCTGCTGTGCTAA
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Results: *P. domestica* and *P. salicina* plums were transformed with the *eIF4E* hairpin RNAi constructs with the aim to silence one or the other isoform and test for resistance to PPV. Similar hp-RNAi constructs are under completion, based on the *eIF4G* and *eIFiso4G* copies. This study is expected to provide clue on the translation initiation factors relevant for PPV, that should be later targeted for resistance to sharka disease.