



Search for natural variants of genes coding
eukaryotic initiation factors in *Prunus* species:

Identification of new sources of resistance to Sharka disease

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Sources of resistance in *Prunus* ?



No true immunity to PPV infection described up to now

Few North-American cultivars are resistant, all genetically related



All susceptible at least to PPV-M, use of a wild, peach-related specie, *Prunus davidiana* to introduce resistance but still, one single source of resistance

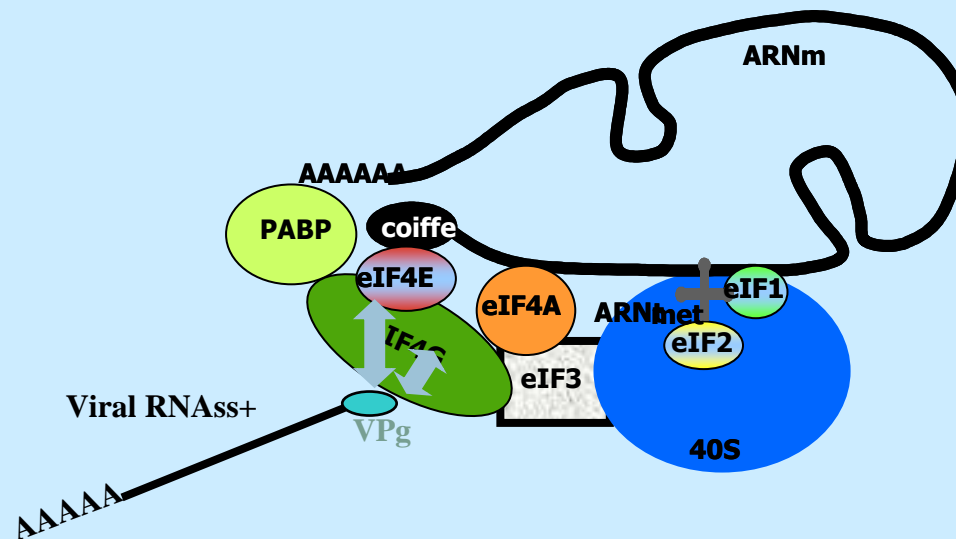


Find new sources of resistance...

Strategy : identify rare alleles of candidate resistance genes in *Prunus* germplasm and/or in wild species

Which candidate genes ?:

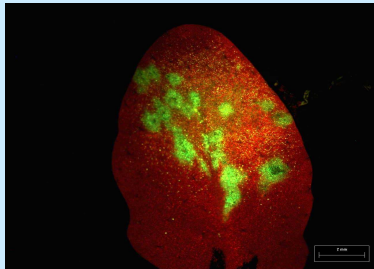
- **Recessive resistance genes:** more than 40% of potyvirus resistance genes are recessive
- Genes involved in the **eukaryotic initiation factor 4F complex (eIF4E & isoforms)**



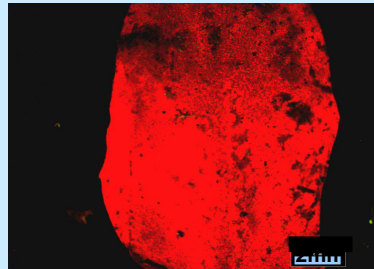
Functional copies of the *eIFiso4E* and *eIFiso4G1* genes are indispensable for PPV infection in *Arabidopsis thaliana*

Inoculation with pBINPPVnkGFP of

Wildtype Col-0



Mutant KO *eIFiso4E*

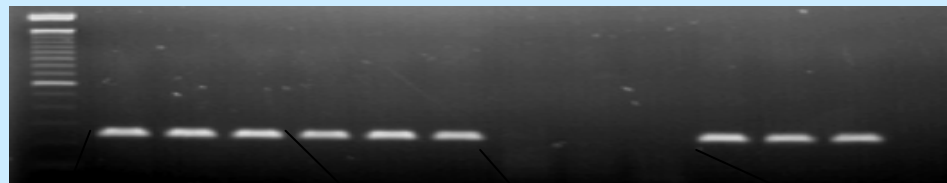


WT

4G

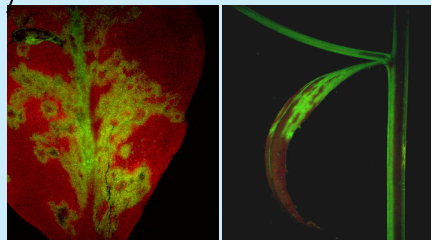
iso4G1

iso4G2



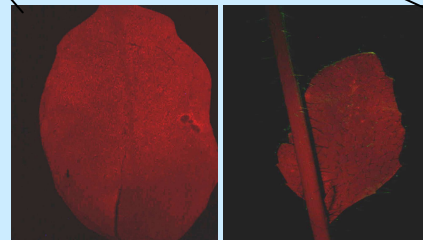
local

distant



local

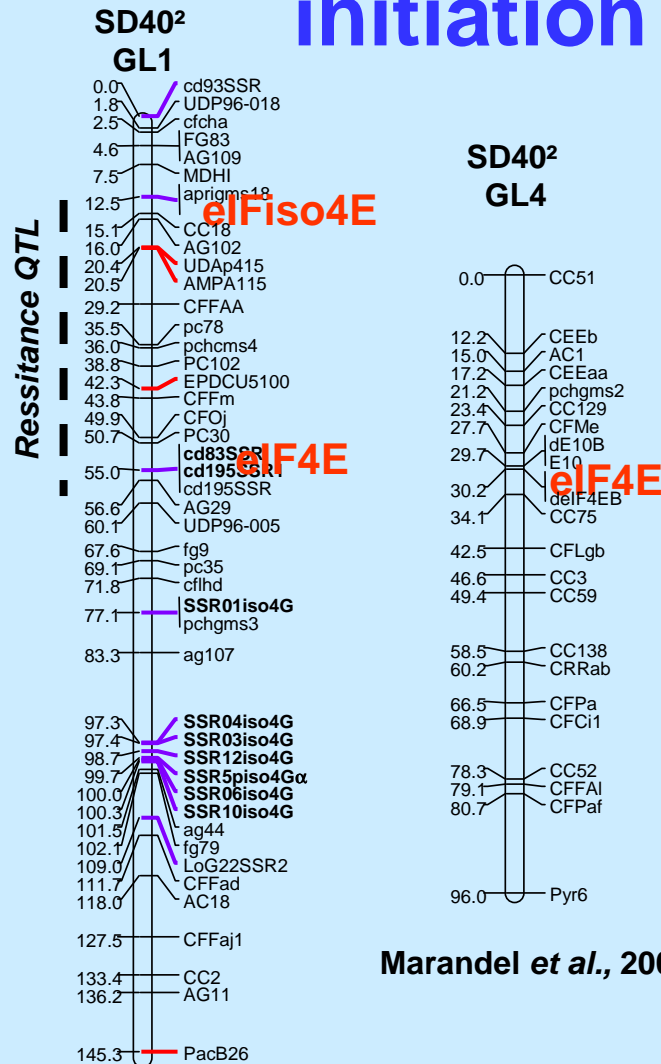
distant



Targeting eIF4E and eIF4G and isoforms for resistance in *Prunus*

Arabidopsis thaliana / PPV:
PPV : eIF(iso)4E (Decroocq et al., 2006)
PPV - eIFiso4G1 (Nicaise et al., 2007)

Prunus orthologs of the translation initiation factor eIF4E



> eIF(iso4E) : only one copy in the *Prunus* genome colocalized with the resistance QTL interval

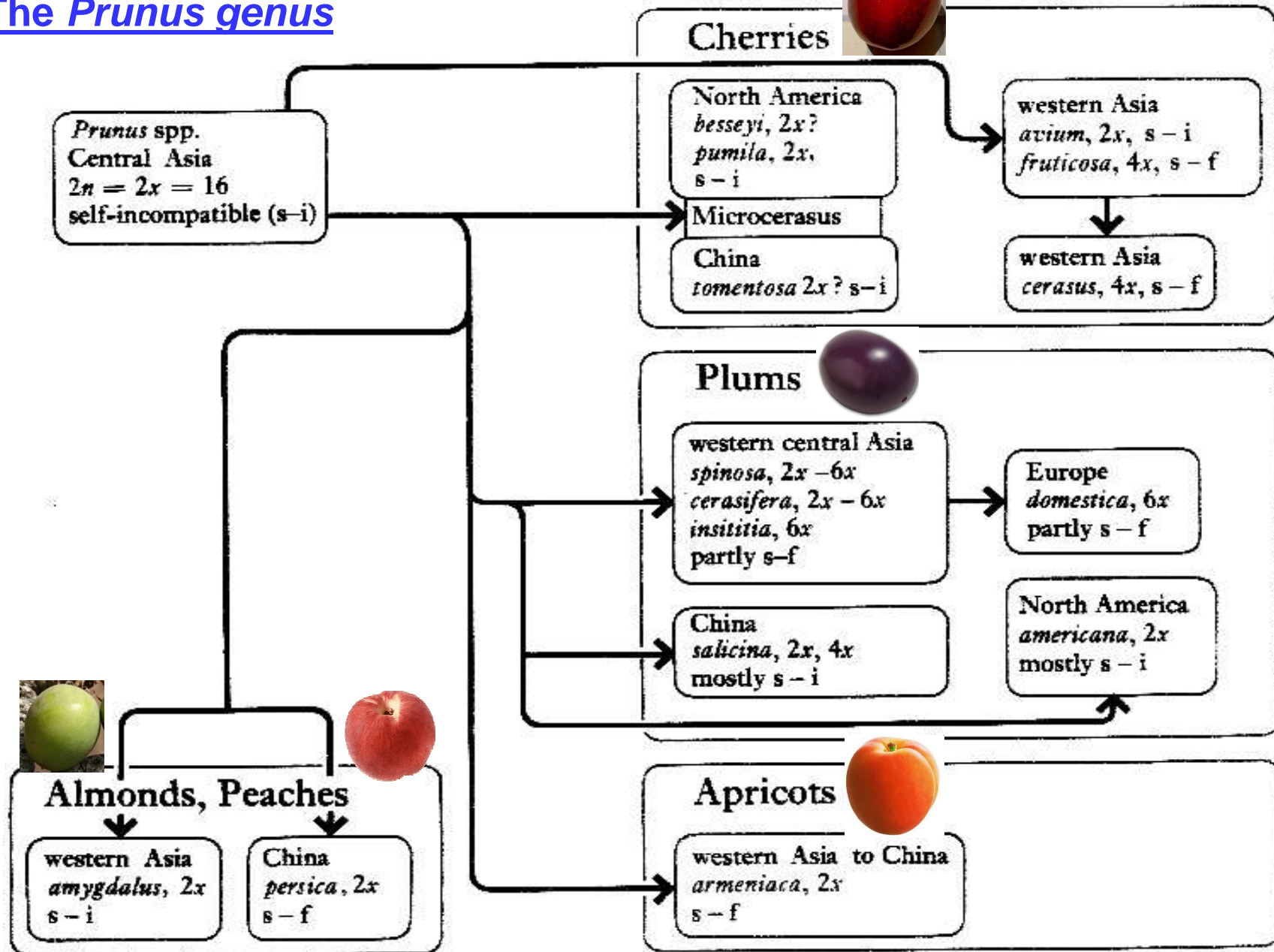
> eIF4E : 2 copies one on GL1 (not functional) & one on GL4

iso4E & eIF4E =
good candidates for
PPV resistance

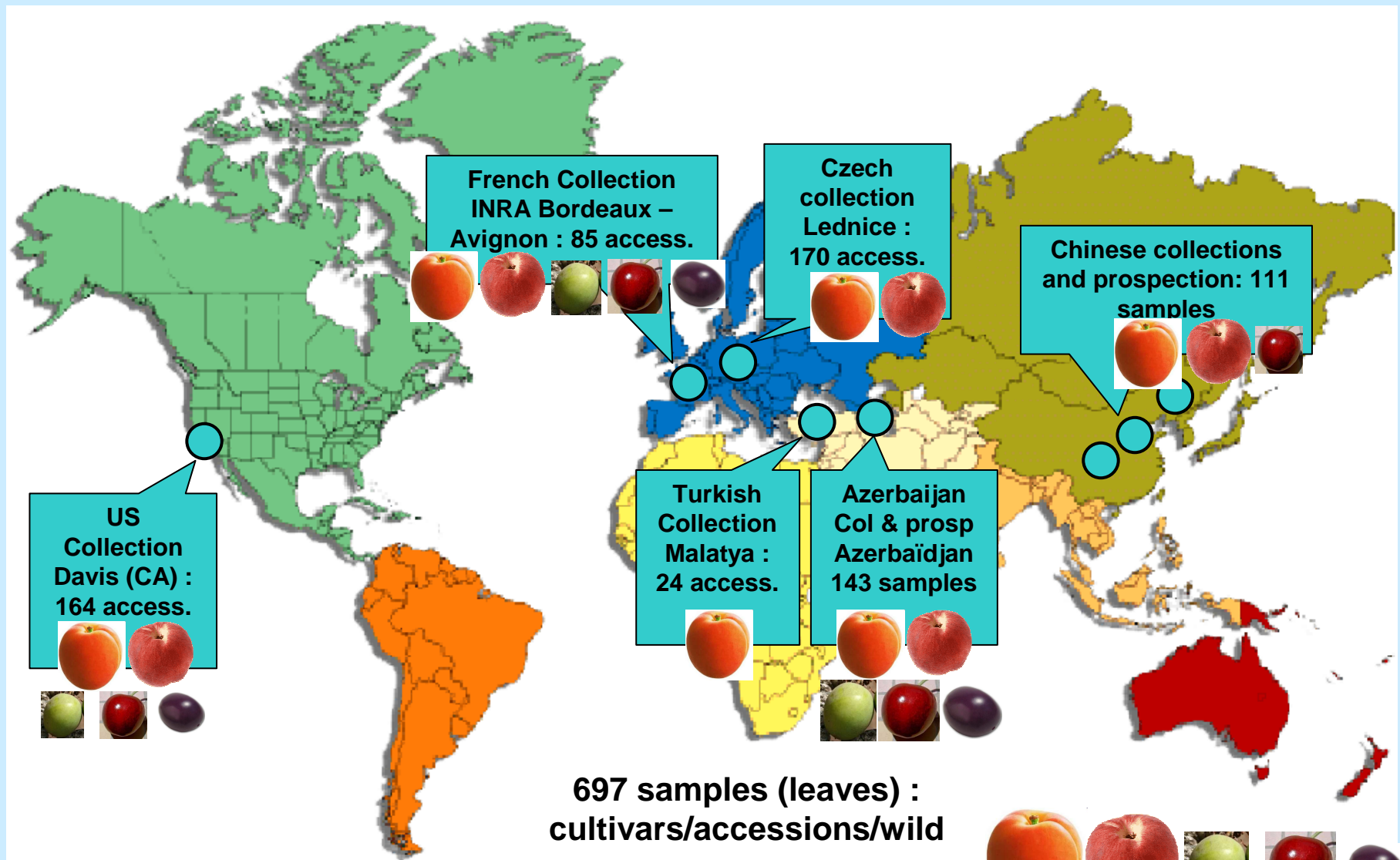
Marandel et al., 2009



The *Prunus* genus

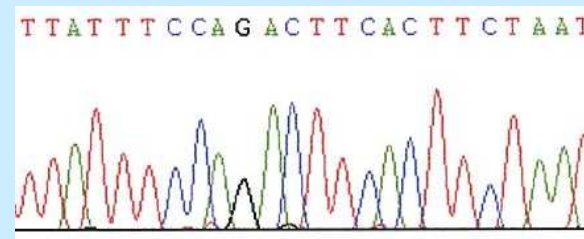


SAMPLING Strategy = cover diversity of germplasm collections then
of material still available in the wild

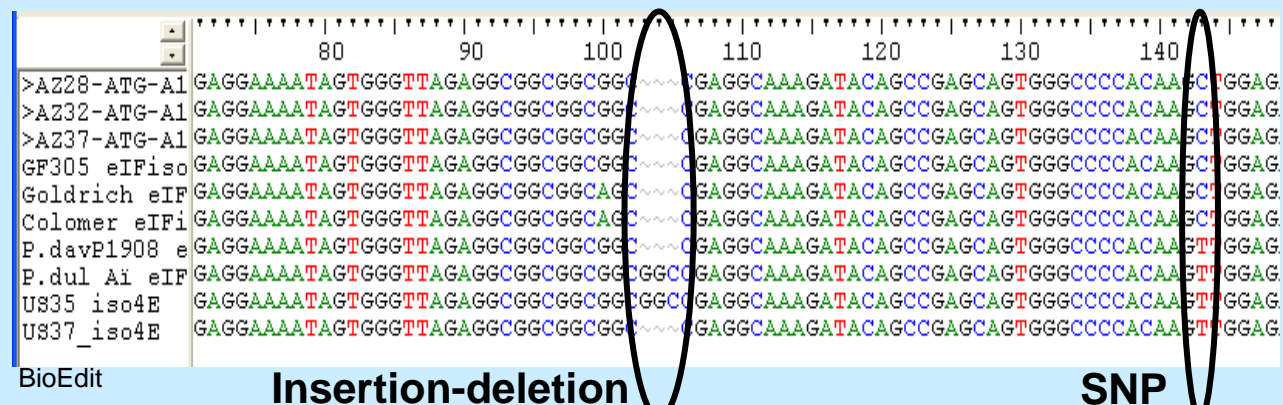
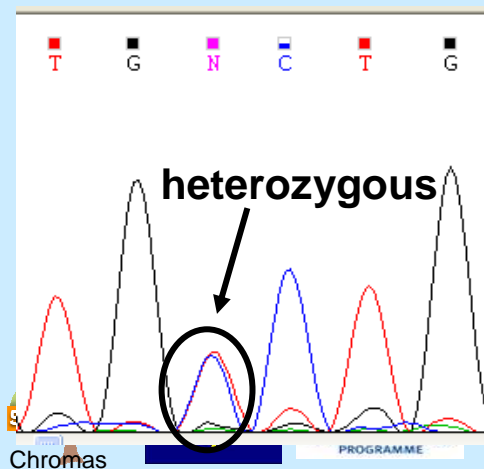


Methodology

- > RNA extraction in 96 wells
- > Reverse Transcription for cDNA
- > Copy-specific amplification eIF4E/(iso)4E
- > Sequencing or HRM analysis

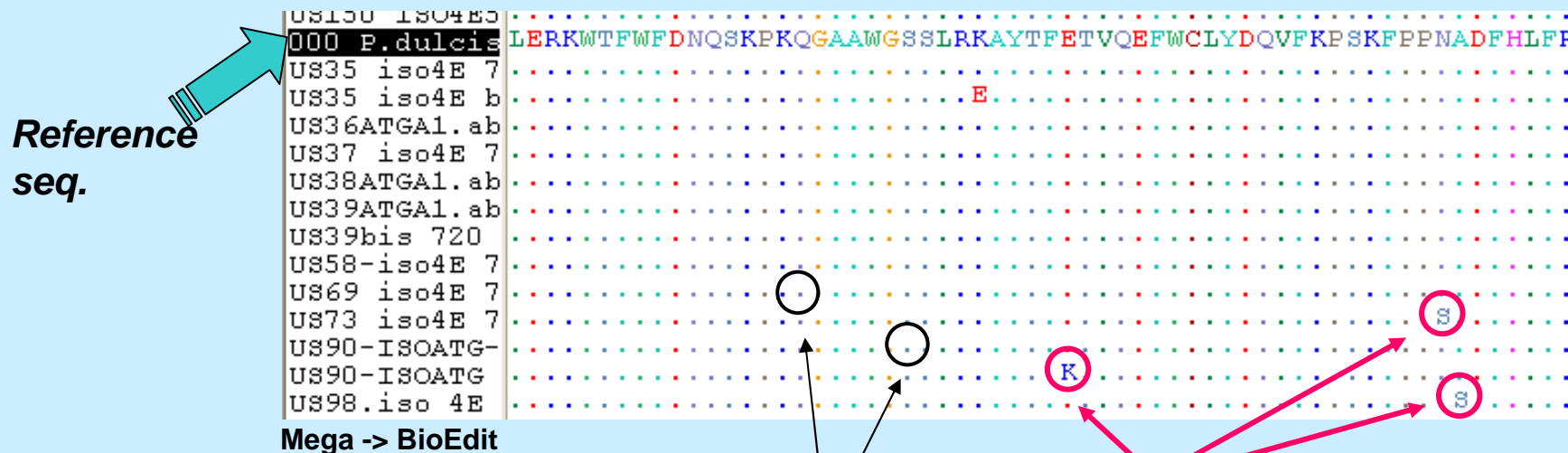


- > Sequence analysis and search for polymorphism



Polymorphism analysis

> Translation: protein sequence



Synonymous mutations

Non synonymous mutations

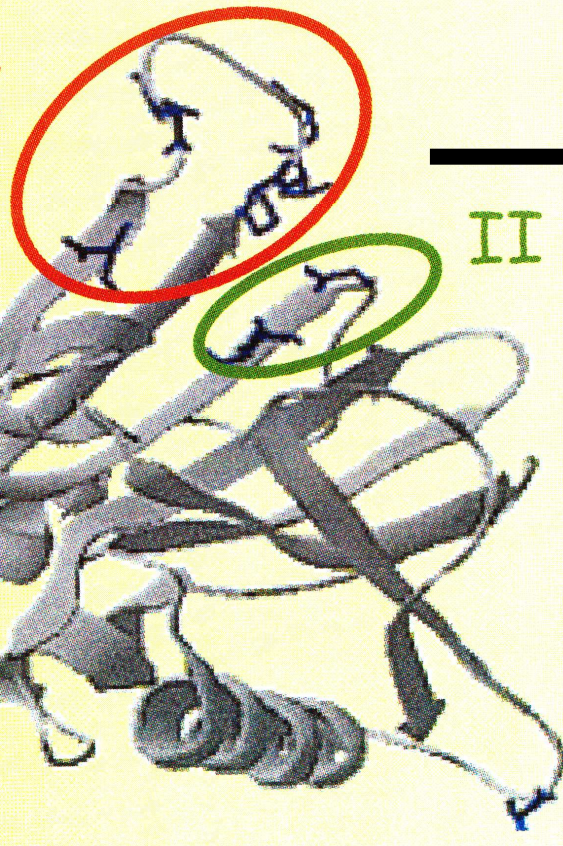
= RARE ALLELES !!!

= SAMPLES TO TEST IN GREENHOUSE FOR PPV RESISTANCE



Selection of the mutations at the protein level

*I et II :
Interaction
domains with
the virus*



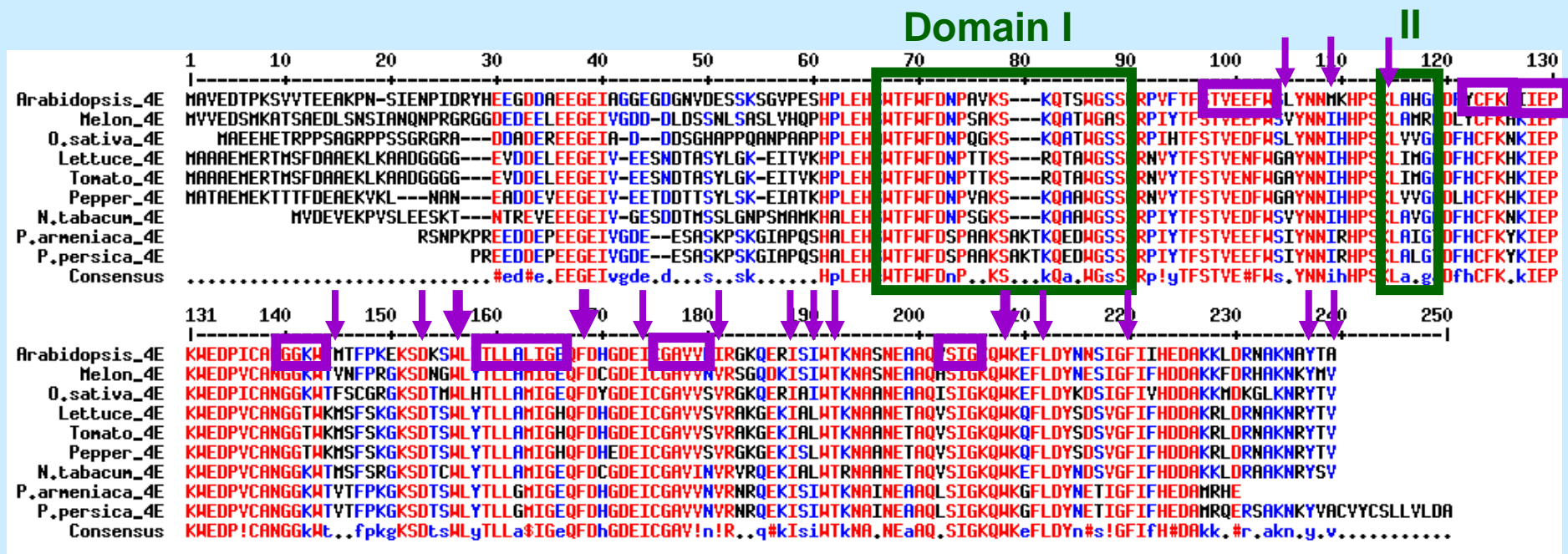
eIF4E protein

1. Target for mutation(s) in the interaction domains

2. Target for mutation(s) predicted to affect the stability of the protein

Example for *elF4E* gene:

interaction domains & important amino acids for protein stability



I et II : Interaction domains in potyviruses (Robaglia and Caranta 2006)

AA affecting protein stability (Charron et al 2008)



Ecotilling: Selection of rare alleles & mutations to test in priority in greenhouse

1. Mutations in the **interaction domains**
or/and mutations affecting stability of the protein

2a. Mutations linked to **homozygous allele**

3a. Resistance test in high confinement greenhouse

2b. Mutations linked to **heterozygous allele**

**Because
recessive
genes !!!**

3b. Self-pollination or Crossing with another mutant

4b. Resistance Test in high confinement greenhouse



Results, based on 700 individuals screened for mutation

eIF(iso)4E : type of mutations observed

Subgenus	Mutation in the interaction domain	Mutation with predicted effect	Mutation with predicted effect but heterozygous
<i>Almond</i>	3	2	0
<i>Apricot</i>	1	2	2
<i>Peach</i>	2	3	2
<i>Plum</i>	1	1	2
<i>Cherry</i>	0	0	1



Grafting in september 2010 for others

Self-pollination



In test for the moment in greenhouse at Bordeaux



eIF4E : type of mutations observed

Subgenus	Mutation in the interaction domain	Mutation with predicted effect	Mutation with predicted effect but heterozygous
<i>Almond</i>	0	4	2
<i>Apricot</i>	0	3	4
<i>Peach</i>	0	1	0
<i>Plum</i>	0	3	3
<i>Cherry</i>	0	25	7

Grafting in september 2010 for others

Self-pollination



In test for the moment in greenhouse at Bordeaux

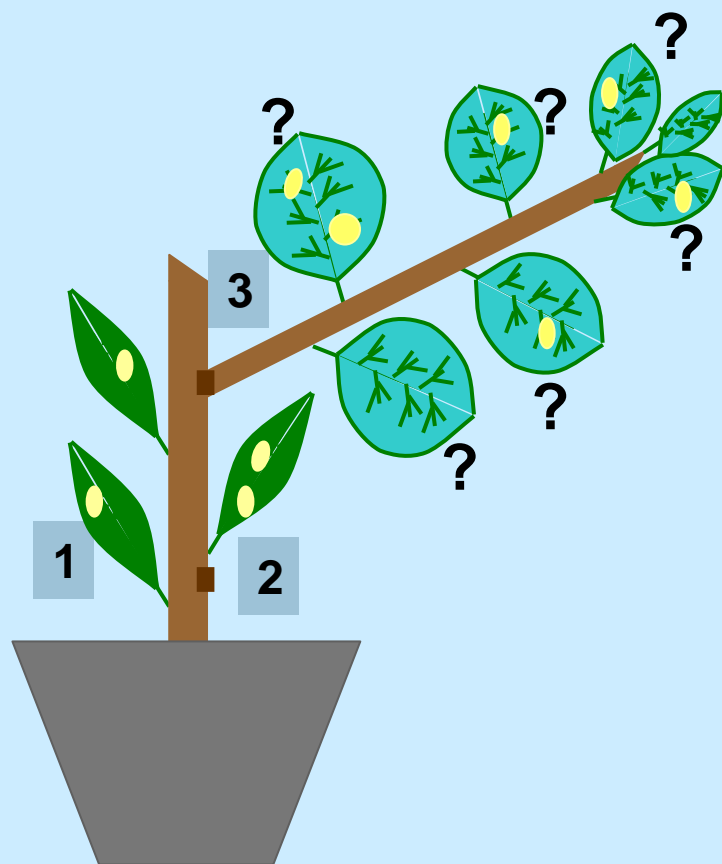
Selection of rare alleles over 700 individuals screened :

eIF4E: 85 rare alleles -> 52 selected for resistance test in greenhouse

eIF(iso)4E 64 rare alleles -> 23 selected for test in greenhouse



Testing resistance to PPV among accessions bearing rare alleles diverging from 'GF305'



Methodology:

- 1 Susceptible rootstock
- 2 PPV infection of the rootstock
- 3 Grafting of the accession to test only on rootstocks showing symptoms

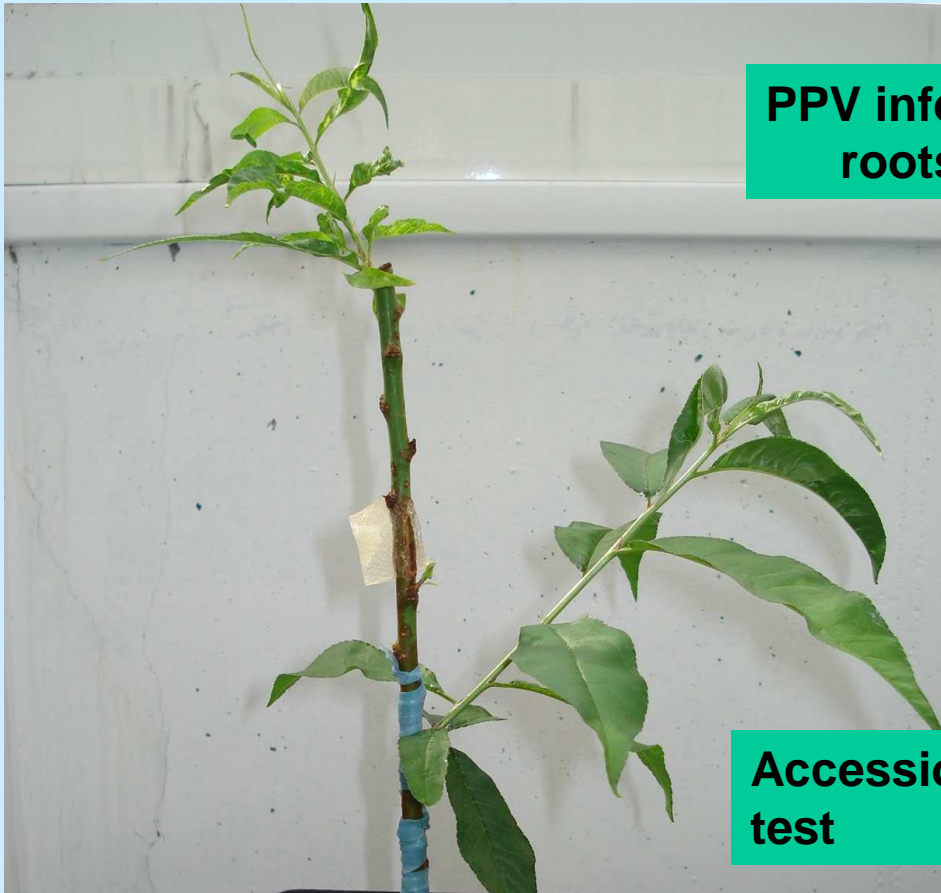
Phenotyping:

- Search for PPV symptoms on leaves
- Viral detection: ELISA and/or RT-PCR

3 observation cycles to confirm or not the behaviour of the virus in the selected individuals



Validation of the rare important mutations



**PPV infected
rootstock**



**Accession to
test**



Results after the 1st cycle :

- 3 accessions with no symptoms (peach –almond) -> ELISA & RT-PCR negative**
- 3 accessions with symptoms**
- 17 accessions on the way to be tested in 2011**

2nd cycle to confirm



Sub-genus	Nb Seq	<i>eIF4E</i>	<i>eIFiso4E</i>
		<i>Nb seq mutated</i>	<i>Nb seq mutated</i>
<i>Peach</i>	205	6.3%	5.5%
<i>Apricot</i>	187	22.5%	12.1%
<i>Almond</i>	55	32.7%	29.4%
<i>Plum</i>	34	47.1%	21.9%
<i>Cherry</i>	40	92.5%	75.6%

Up to 95% of the sequences obtained are grouping with the reference sequences, means that they bear alleles of susceptibility

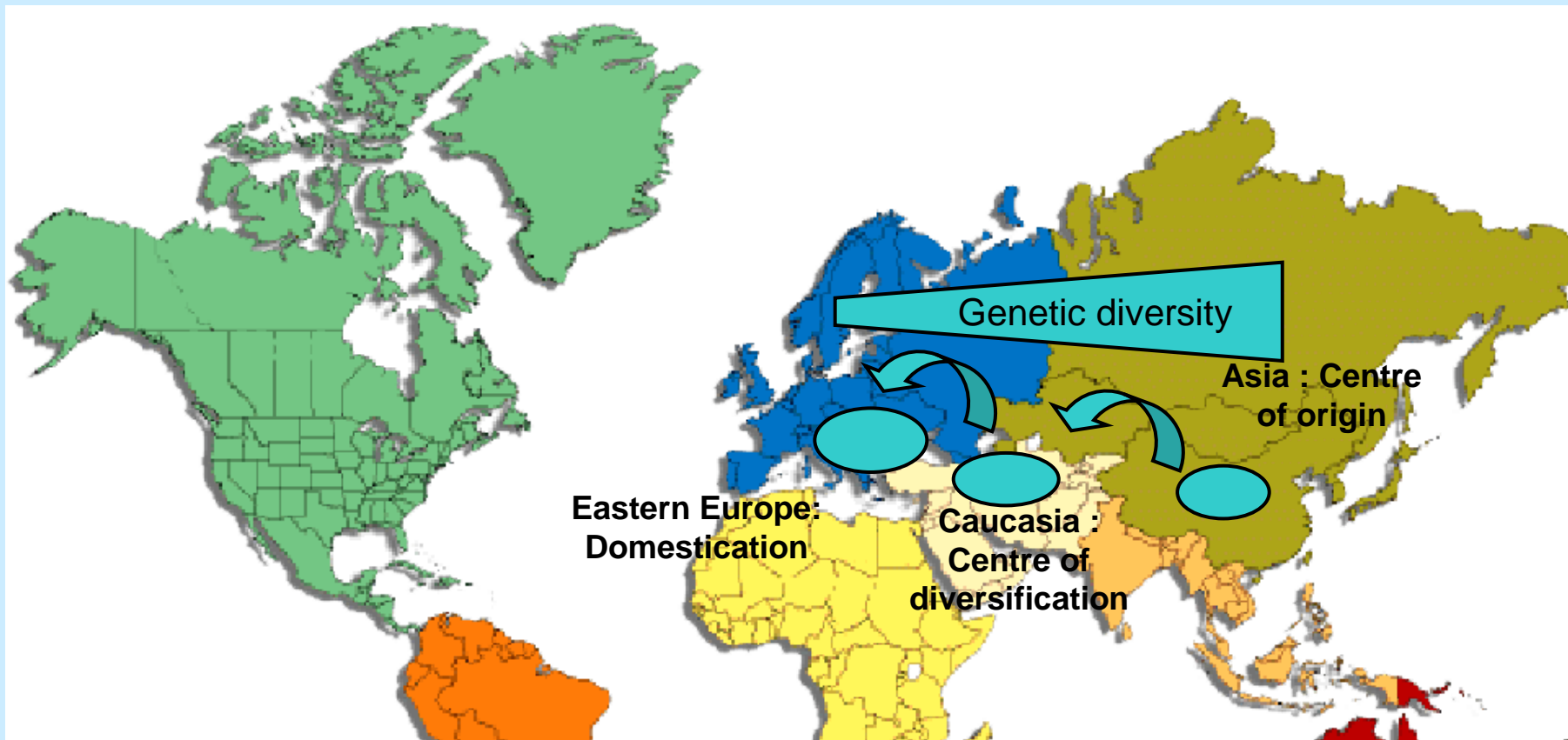
For over 200 peach accessions screened, only 13 to 10 haplotypes differing from 'GF305', means very few diversity in peaches

For about 180 apricot genotypes tested, more diversity identified : 42 to 21 haplotypes

A majority of rare haplotypes are issued from Asian material, the most interesting are wild original plants



Prunus : Evolution within the cultivated *Prunus* species



Screening extended through STONE consortium between China, Caucasian and European countries (IRSES Marie Curie project 2011-2014, under negotiation and coordinated by V. Decroocq)



Perspectives...

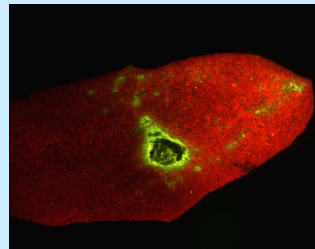
Screen natural diversity for other candidate genes of the translation initiation complex : eIF4G and isoform

but in multi-copies, needs to adapt the strategy

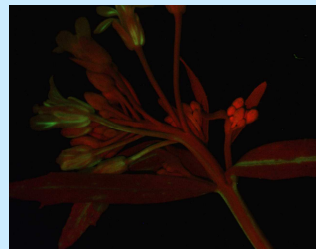
Identification of new genes of recessive resistance to PPV on *Arabidopsis thaliana* (see poster Pagny et al.) and transfer to *Prunus*

KO mutation

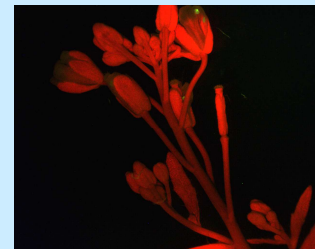
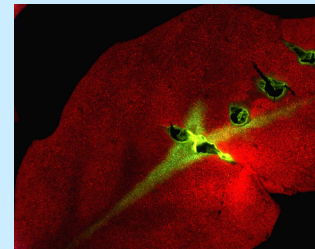
Inoculated leaf
9 dpi



Floral hamp
21 dpi



Columbia



Development of a collection of mutagenised peach 'GF305' and screening for mutations in the above candidate genes.

