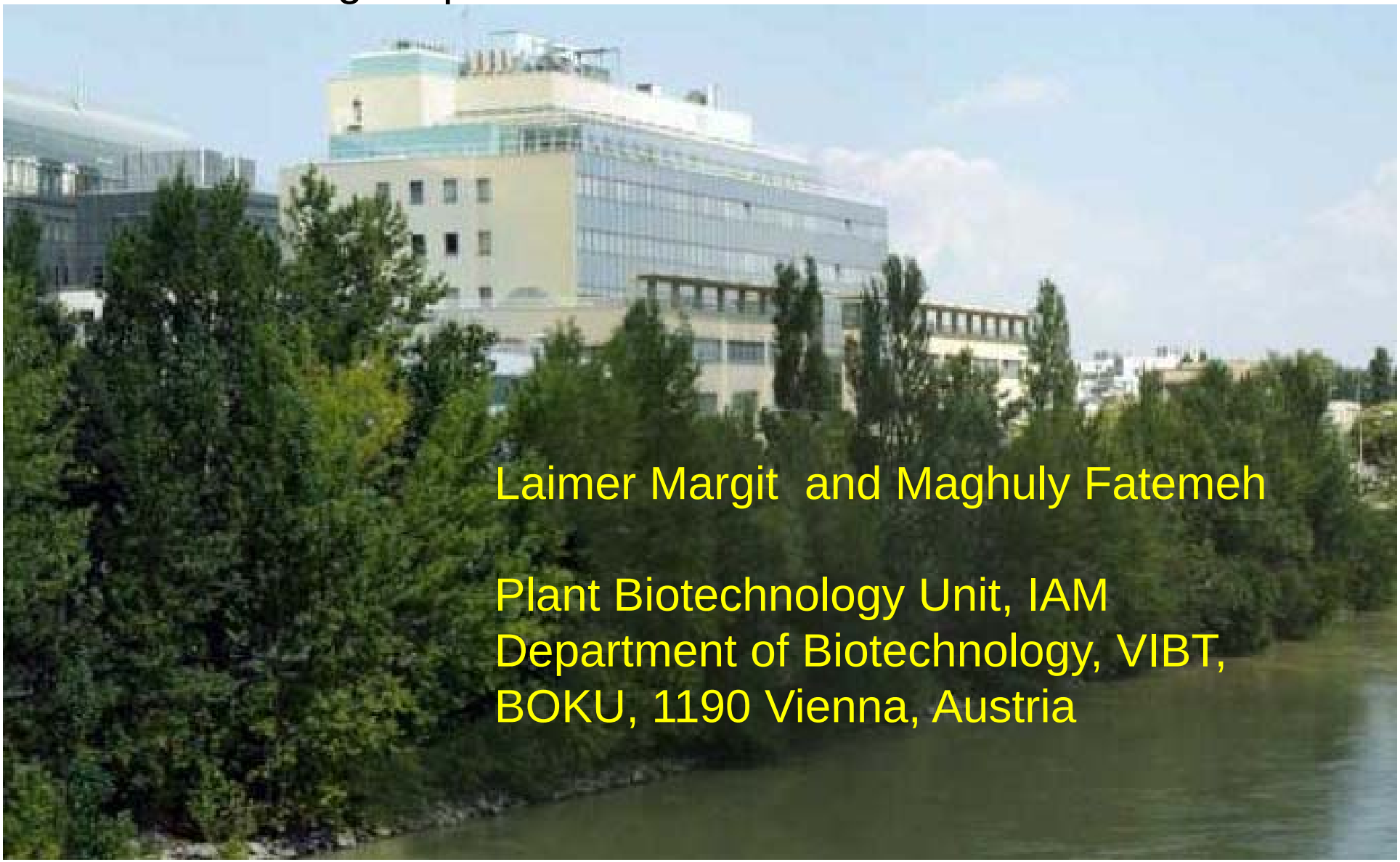
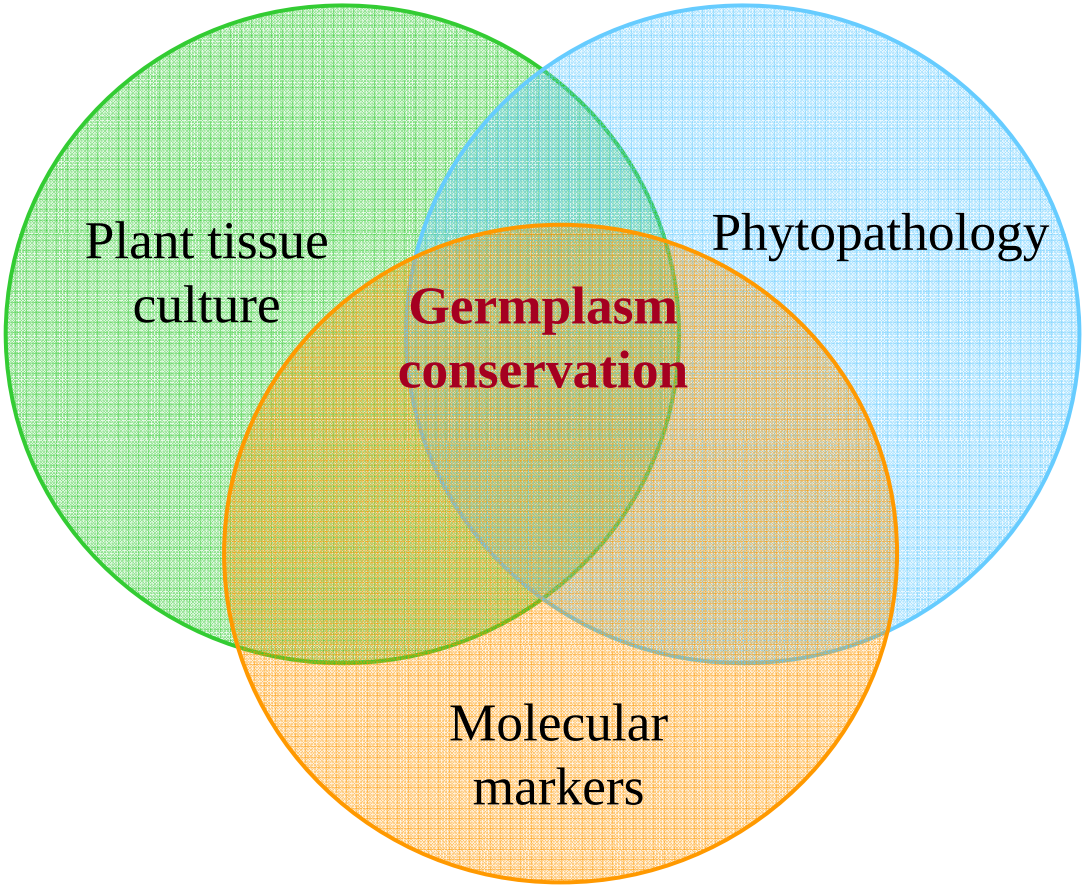
Contributions of biotechnological tools to the conservation of valuable germplasm



Laimer Margit and Maghuly Fatemeh

Plant Biotechnology Unit, IAM
Department of Biotechnology, VIBT,
BOKU, 1190 Vienna, Austria

Biotechnology: a transdisciplinary approach



Challenge of conservation

Purpose of conservation for current and future use

(remember, our life depends on very few domesticated plant species)

Challenge of conservation

Selection of accessions for conservation (sufficient variability versus unnecessary costs)

Challenge of conservation

Quality assessment of conservation (is the conserved population still vital under natural conditions?)

in situ

ex situ

in vivo

in vitro

Regeneration practices

- objective: to regenerate while maintaining accession integrity

- practices differ
  - from genebank to genebank
  - from crop to crop
  - from species to species within a crop complex
  - from accession to accession
  - from environment to environment

Micropropagation

ACCLIMATIZATION MOTHER-PLANT

ROOTING

MULTIPLICATION

INITIATION

Vaccinium genebank in vitro

V. corymbosum	10 cultivars
V. myrtillus	20 accessions
V. cylindraceum	25 accessions

Cryo-conservation

Storage at - 196° C in liquid N_2

- requires continued technical surveillance
- allows storage of *in vitro* buds up to 10 years

Challenges of pathogens to collections of genetic resources

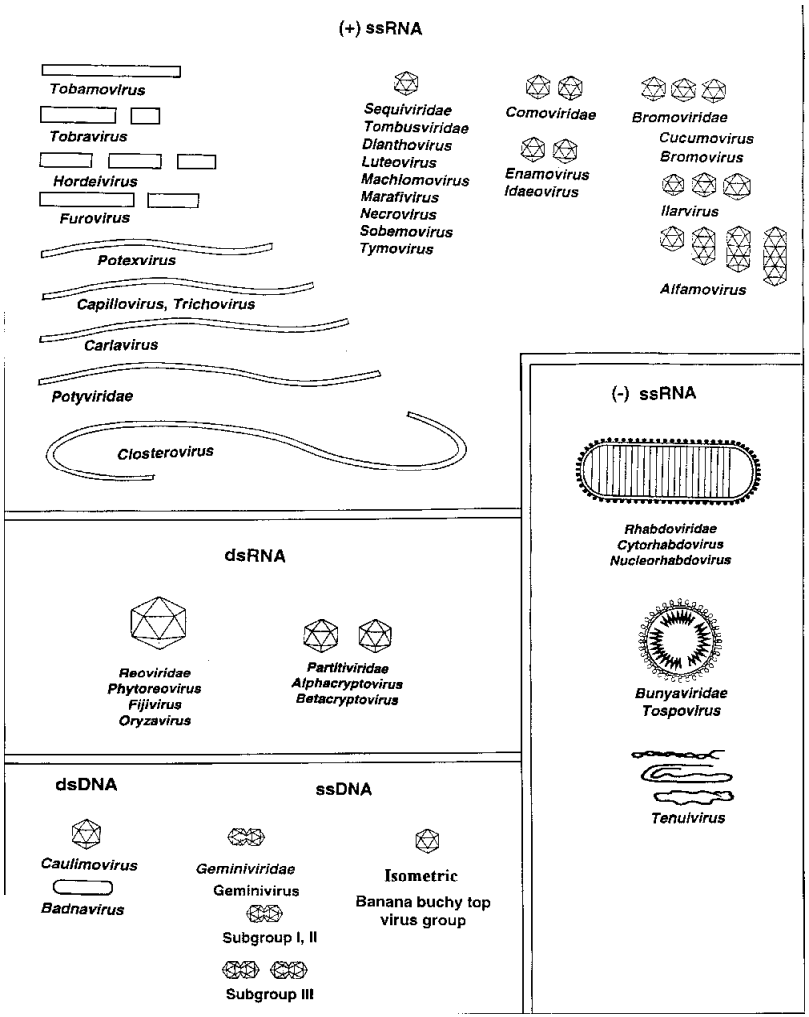
- Importance of phytopathological aspects in botanical collections:
  - Vicinity of new neighbors
  - New vectors
- High impact on conservation of valuable genetic resources
  - Sudden or earlier death of infected material
- Morphological traits might be severely impacted by e.g. virus infections
  - GFLV leaf symptoms
  - Stunting symptoms
  - Mosaic symptoms

Additional effects of globalization

- Movement of plants
- Movement of vectors
- Movement of pathogens

Losses to viruses and phytoplasmas

rank second worldwide

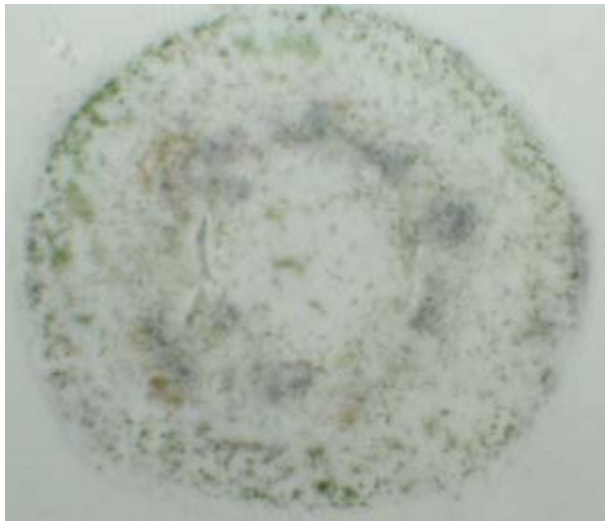


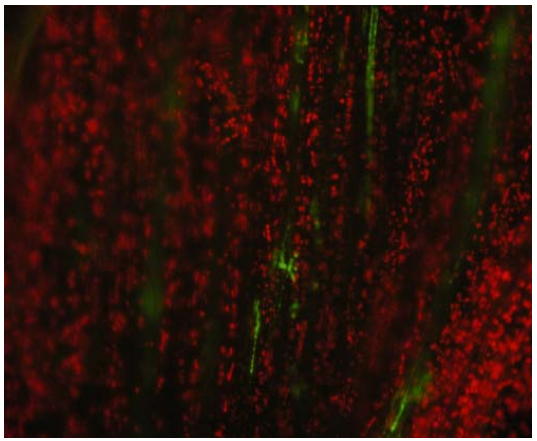
Serological detection of pathogens

ELISA Enzyme Linked ImmunoSorbent Assay

ITP Immuno-Tissue Printing

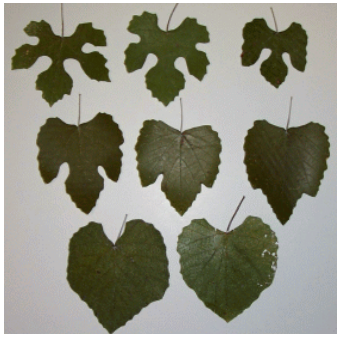
IF Localisation by Imunofluorescence

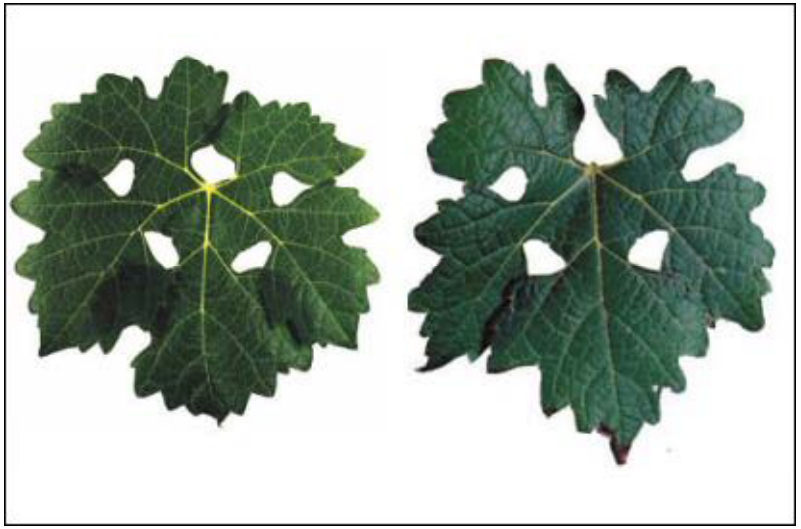




Knapp et al. 1995. J. Virol. Meth. 55:157-173

Viruses interfere with morphology

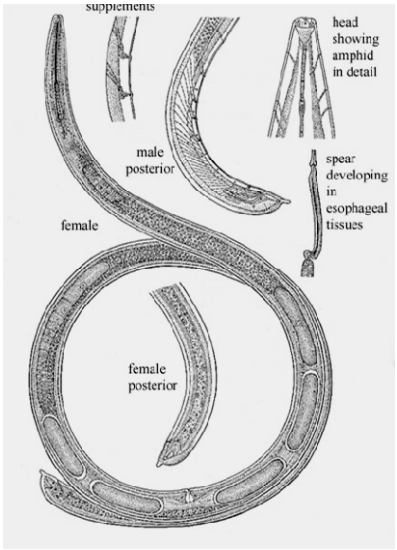




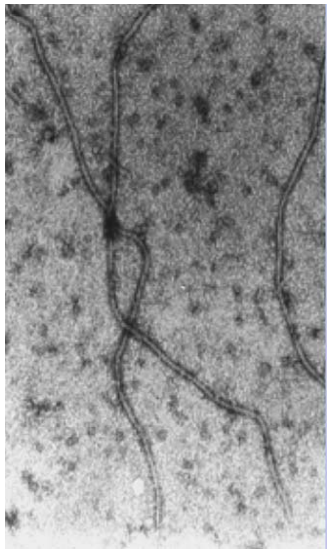
- decrease the vitality of the accession
- interfere with morphological traits

Grapevine fanleaf virus (GFLV)

GFLV
Family *Comoviridae* Genus *Nepovirus*



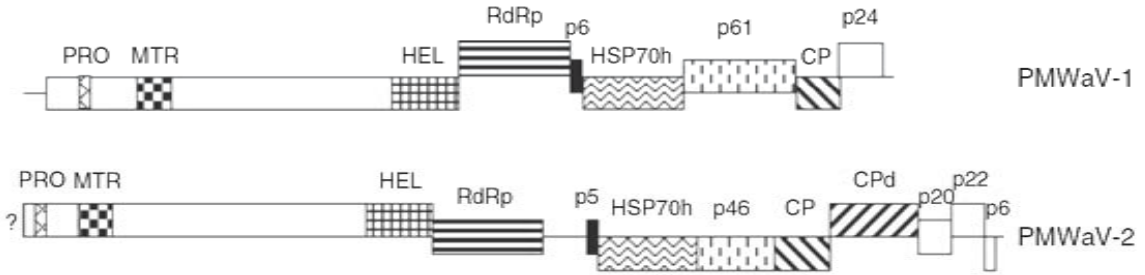
Viruses in vegetatively propagated plants



Mealybug Wilt of Pineapple (MWP)

Reddening of the leaves
Downward curling of the leaf margins
Loss of turgidity, leaves reflex downwards
Leaf tip dieback
Plants either recover or endure further leaf tip dieback resulting in death

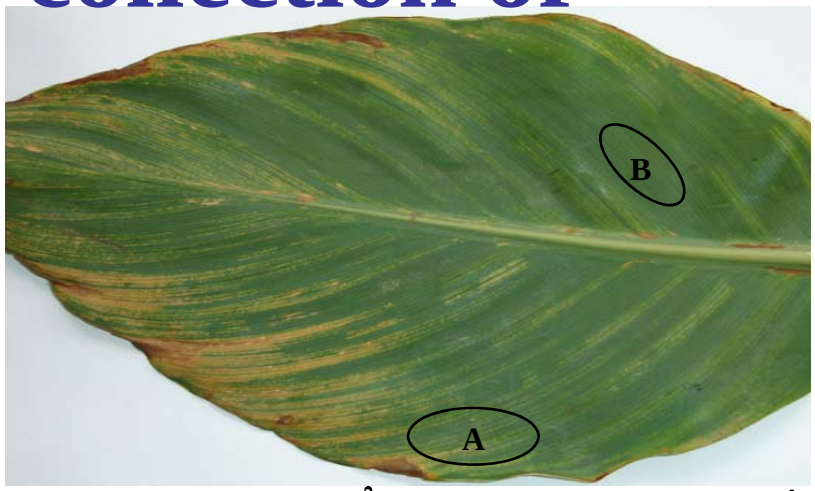
Family *Closteroviridae* Genus *Ampelovirus*



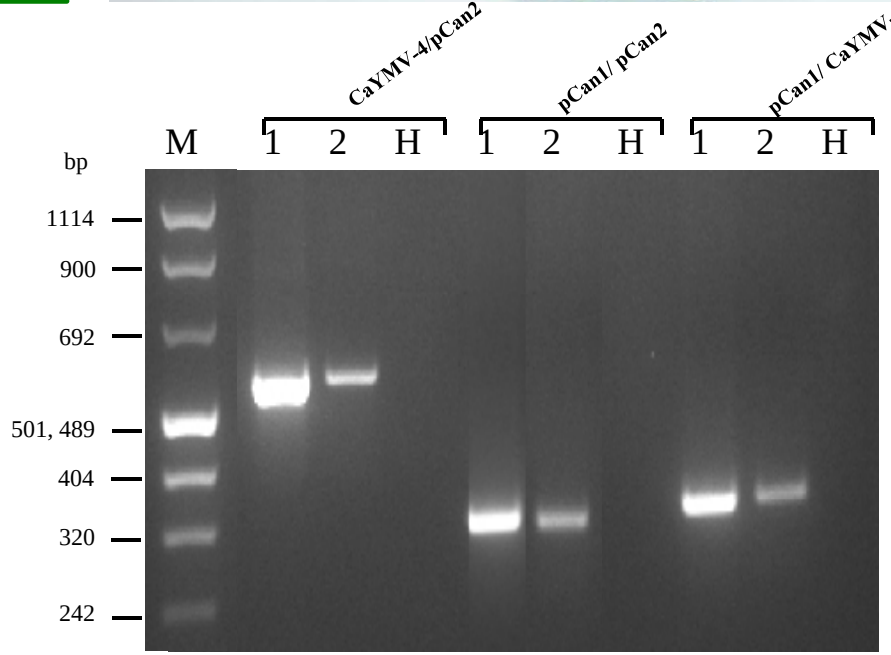
PMWaV-3
PMWaV-4 ?

Canna yellow mottle virus (CaYMV) detection in a cultivar collection of *Canna indica*

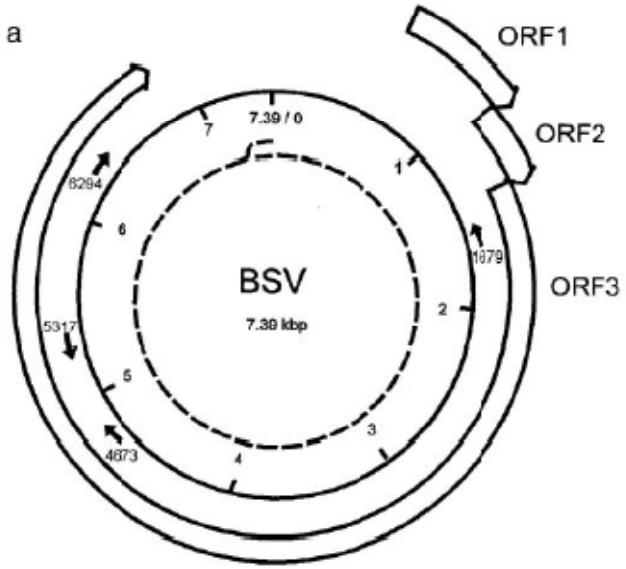
Primer combinations	Expected size	Annealing temperature
CaYMV-3/CaYMV-4	565 bp	60°C
CaYMV-4/pCan2	534 bp	58°C
pCan1/ pCan2	315 bp	55°C
pCan1/ CaYMV-3	333 bp	58°C



Isolates from different cultivars (Perkeo, Lucifer, Opera La Boheme and V17) show a high degree of homology (> 98%), indicating a secondary infection in the collection

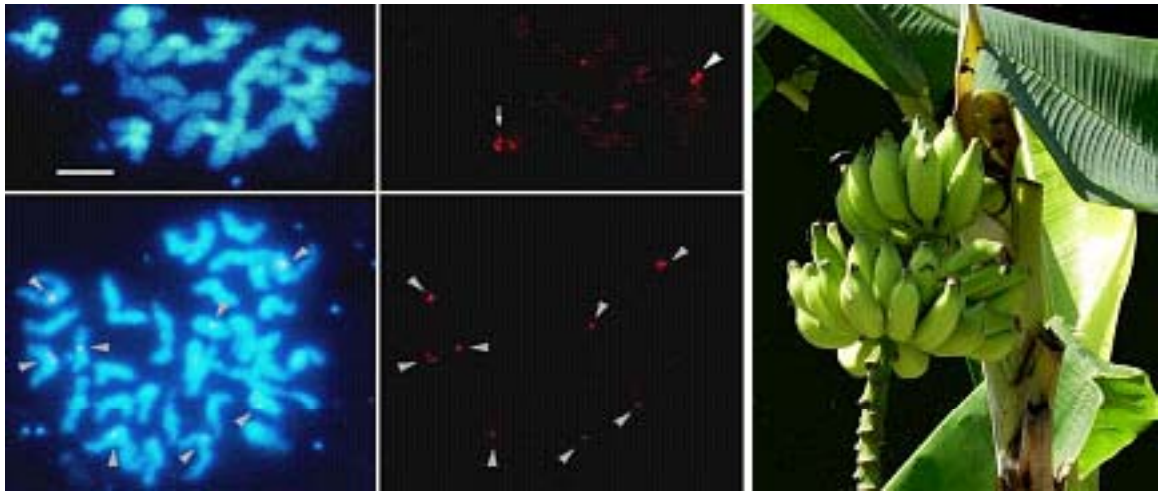


Banana bunchy top virus (Badnavirus) (BBTV)



Banana streak disease is caused by several distinct badnavirus species, one of which is Banana streak Obino l'Ewai virus (Harper et al. 1999). Banana streak Obino l'Ewai virus has severely hindered international banana (*Musa spp*.) breeding programmes, as new hybrids are frequently infected with this virus, curtailing any further exploitation.

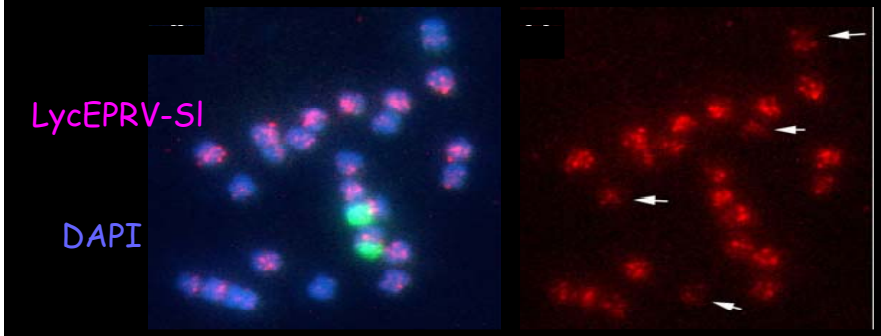
Banana bunchy top virus (Badnavirus) (BBTV)



This infection is thought to arise from viral DNA integrated in the nuclear genome of *Musa balbisiana* (B genome), one of the wild species contributing to many of the banana cultivars currently grown (Geering et al. 2005)

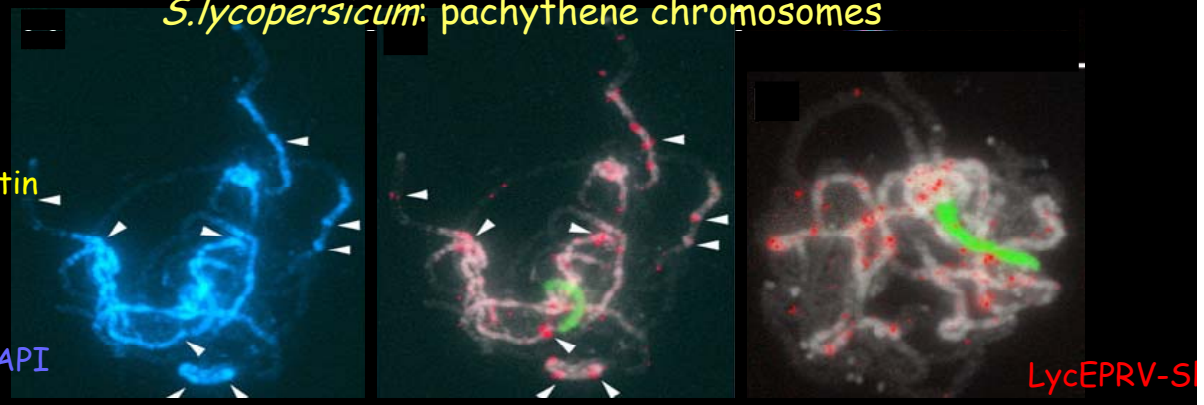
Chromosomal localization of *Lyc*EPRVs

S.lycopersicum: 2n = 24

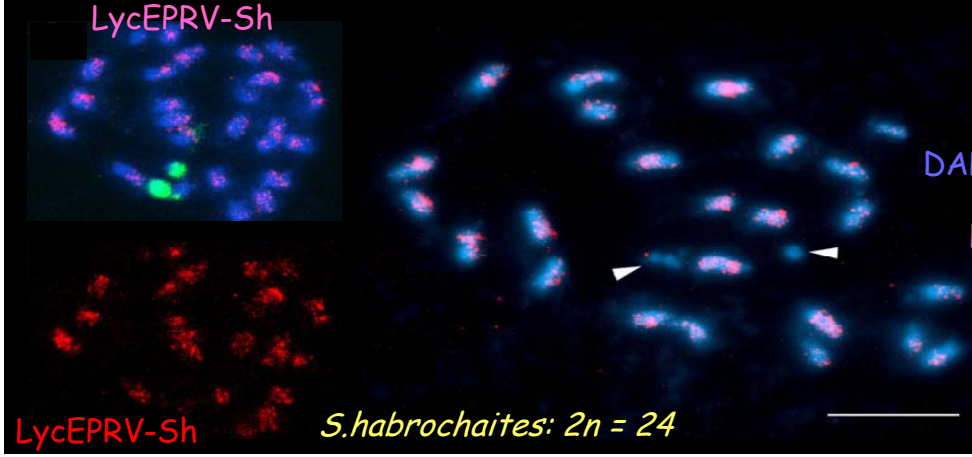


→ pericentromeric
→ reduced on 2 chromosome pairs
→ absent from NOR

S.lycopersicum: pachythene chromosomes



→ *Lyc*EPRV almost exclusively in heterochromatin
→ several sites per chromosome
→ not in NOR



→ pericentromeric, variable intensity
→ excluded from NOR

Staginnus et al. 2008

Methods for pathogen elimination

***In vivo* thermotherapy**

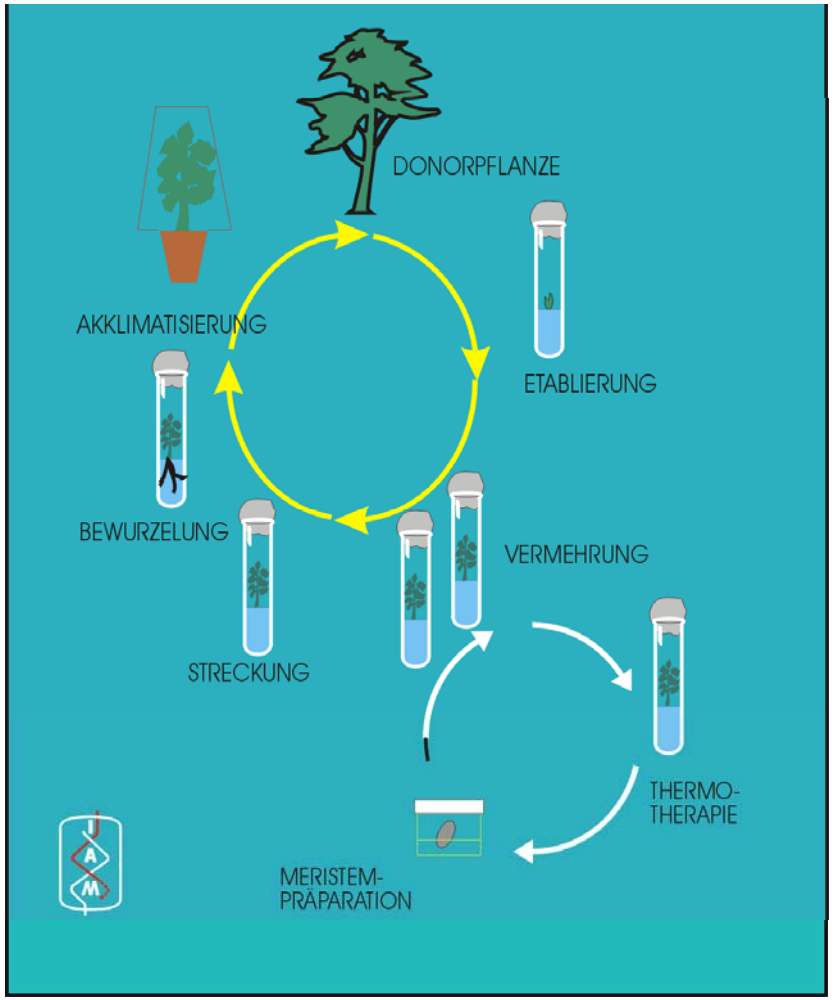
treats 2-year old plants for several weeks

followed by grafting on virus-free rootstocks

IAM-scheme for pathogen elimination

***In vitro* thermotherapy**

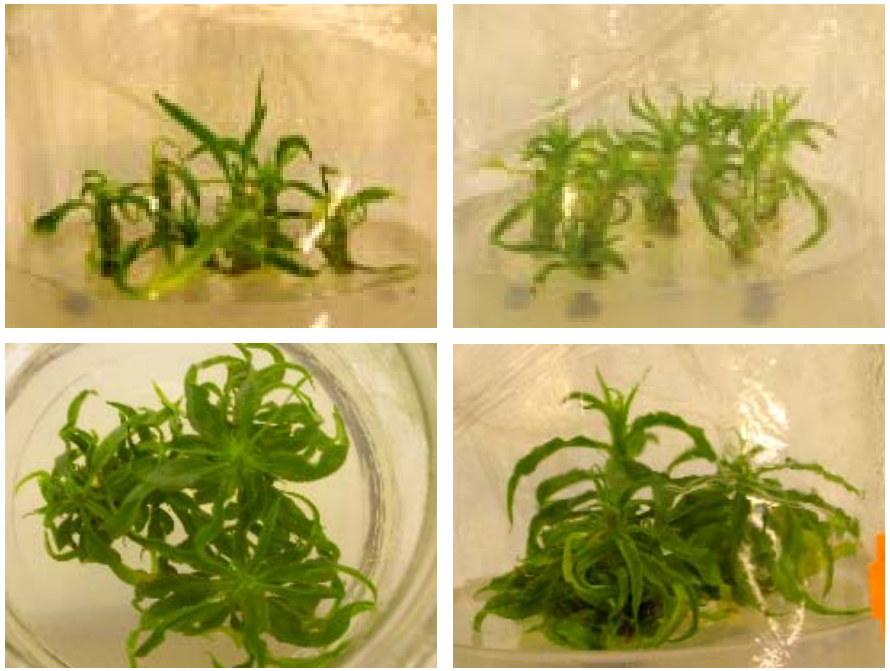
- treatments of *in vitro* cultures at 38°/36°C for 21 days
- meristem preparation and plant regeneration
- optimisation of *in vitro*-culture conditions of thermotherapy and meristem regeneration
- improved detection



Laimer M. 2003. Hort. Reviews 28: 187–236

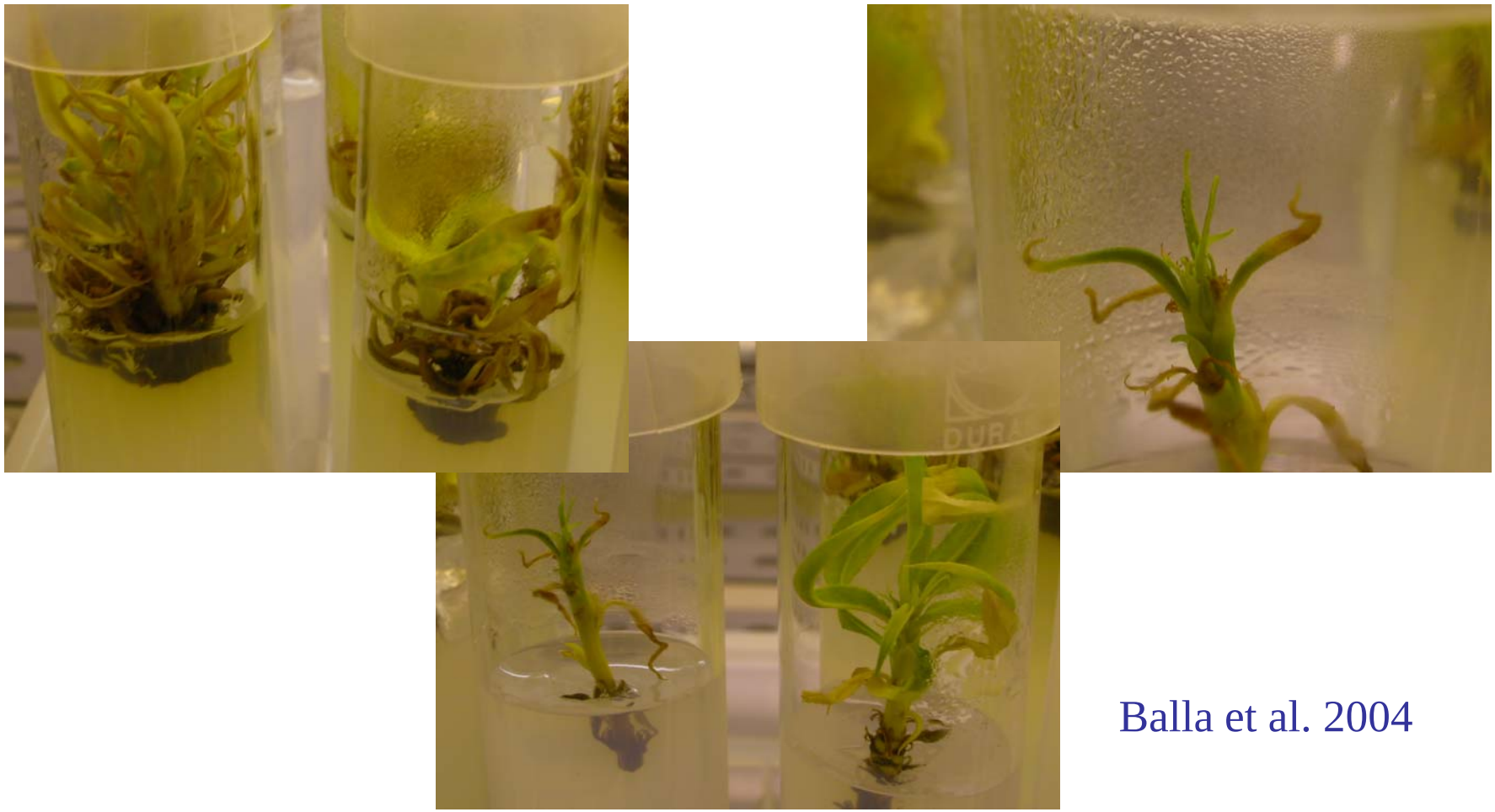
Methods for pathogen elimination

- Multiplication rate of peach shoots *in vitro* may depend on the degree of virus infection



Balla et al. 2004

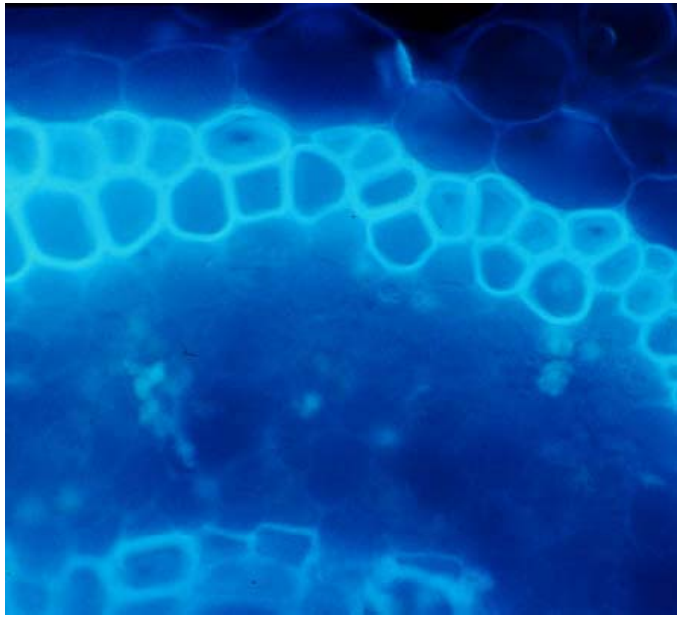
In vitro thermotherapy of *Prunus persica*



Balla et al. 2004

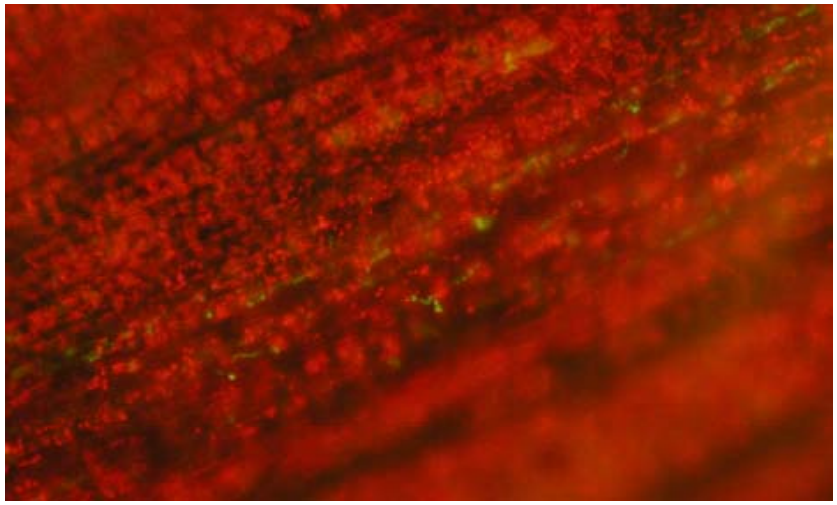
Detection methods for phytoplasmas

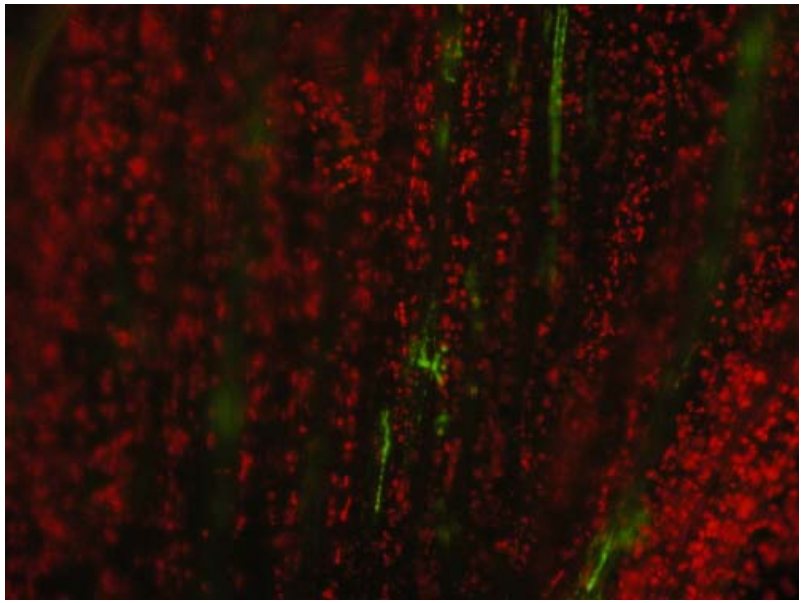
- Indexing on specific host plants
- Fluorescence with DNA dye DAPI (4,6-diamidino-2-phenylindole)



Phytoplasma elimination by *in vitro* thermotherapy and meristem preparation

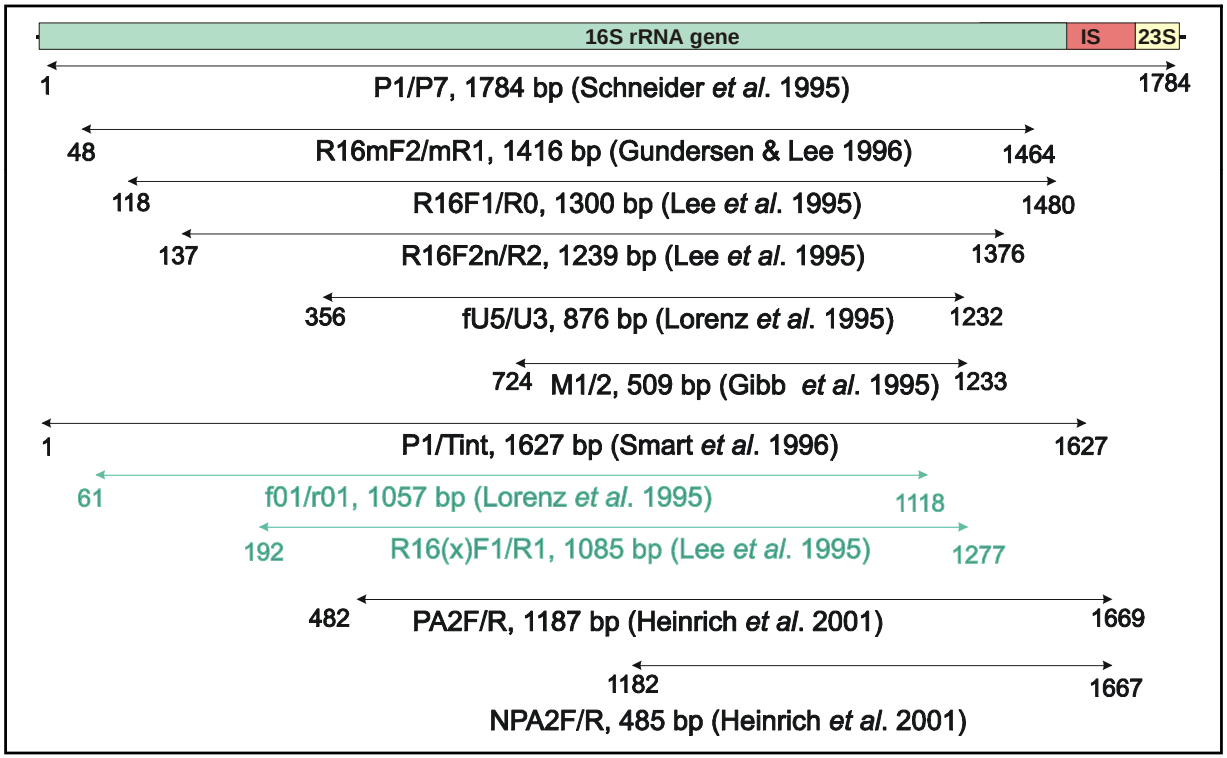
Serological detection: Immunofluorescence allows to localize AP





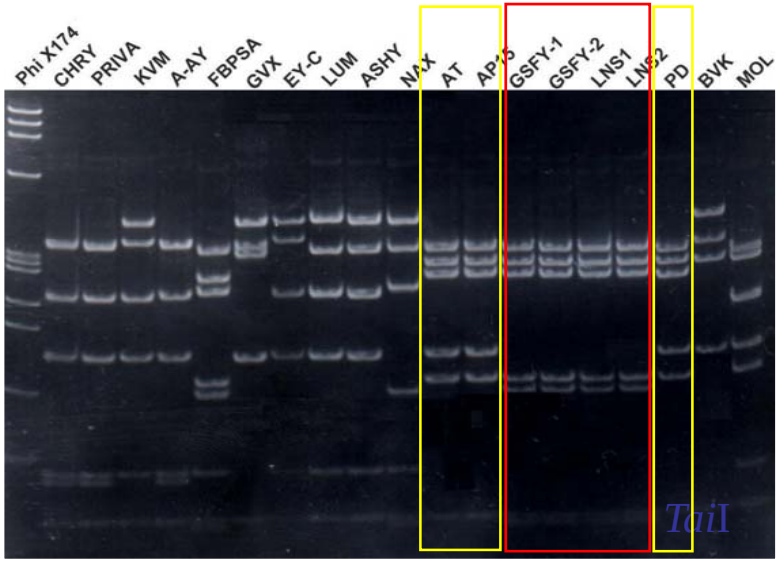
Molecular detection methods for phytoplasmas

- Detection of 16S-DNA with different primers

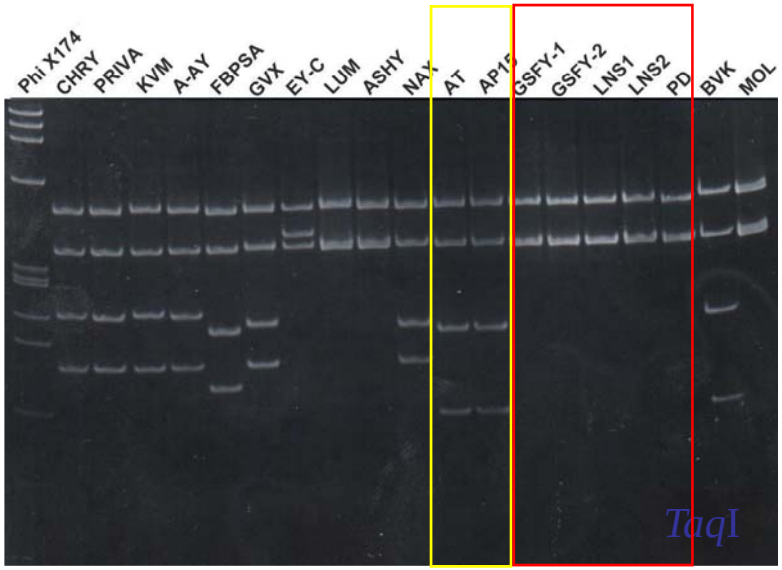


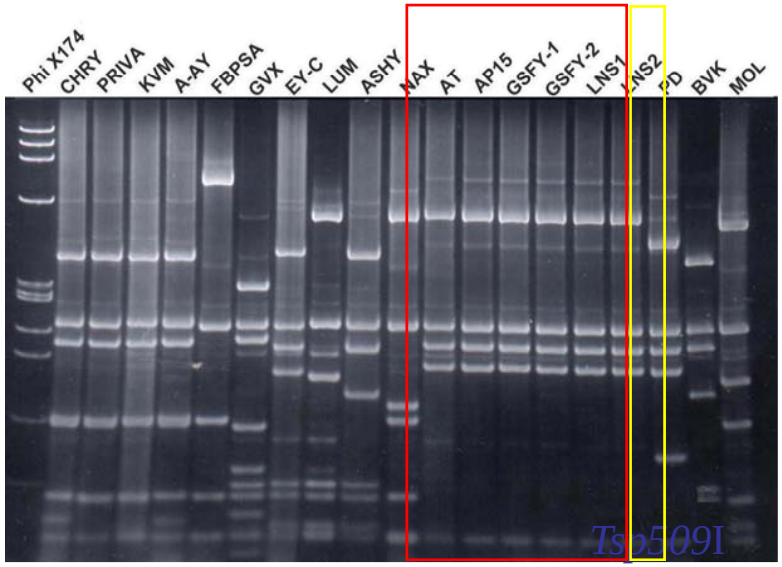
Heinrich et al. 2001. Plant Mol. Biol. Rep. 19:169 -179

Molecular Detection for Phytoplasmas



RFLP analysis of PCR fragments with primers PA2F/R allows the distinction most actually known groups of phytoplasmas





Heinrich et al. 2001. Plant Mol. Biol. Rep. 19: 169-179

Symptomatic plants in the forest


Rubus ideaeus


Betula alba


Fraxinus sp.


Rubus fruticosus

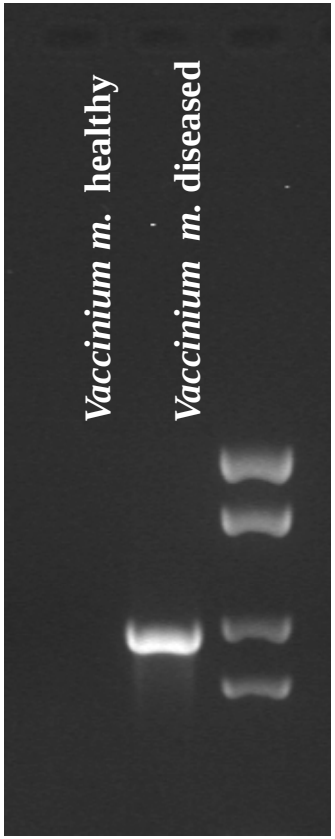

Convulvulus arvensis


Sorbus aucuparia

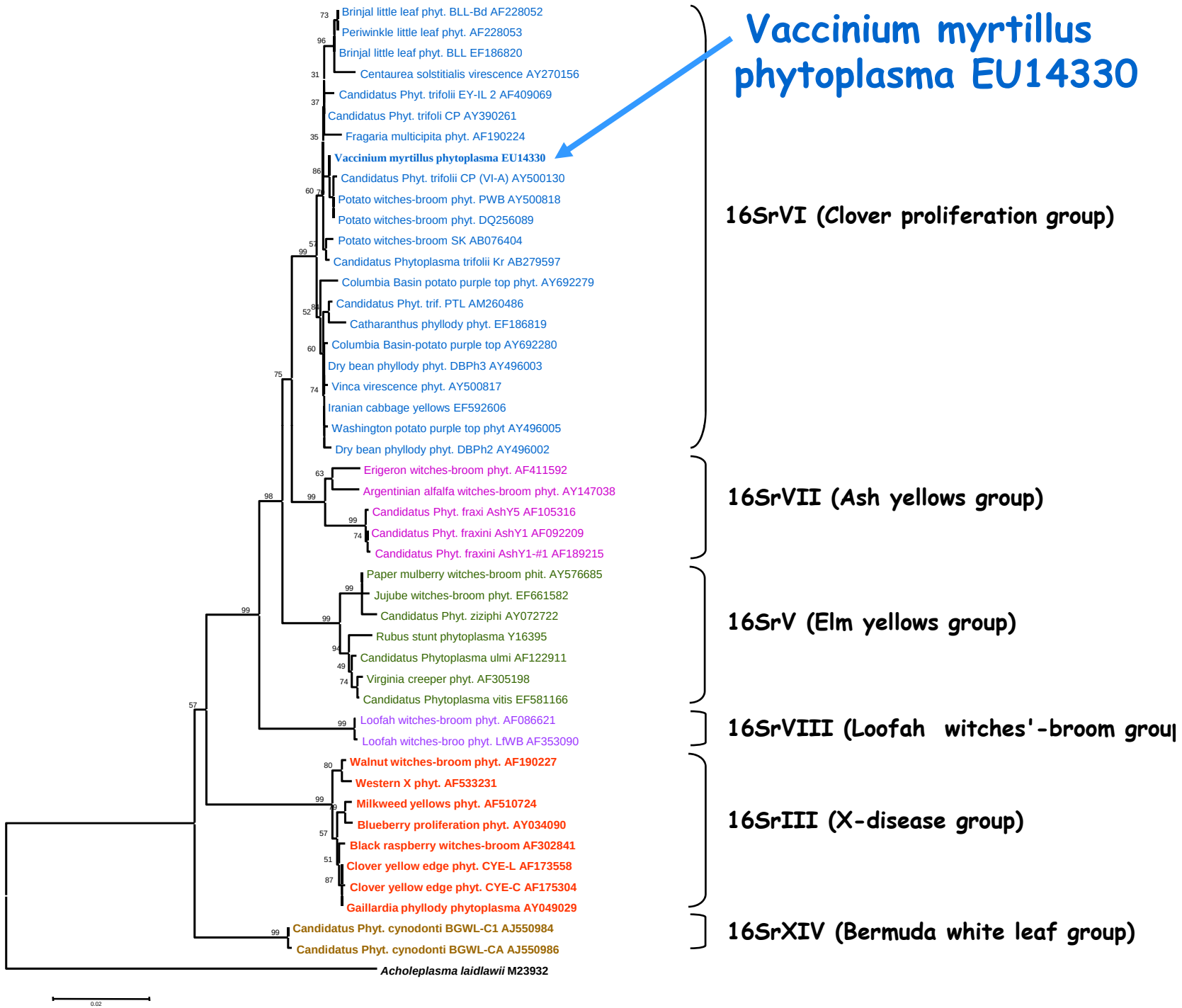

Fagus sp.


Cornus mas

Symptomatic plants of *Vaccinium myrtillus*



16Sr VI as confirmed by sequencing


Vaccinium myrtillus phytoplasma EU14330
Brinjal little leaf phyt. BLL-Bd AF228052
Periwinkle little leaf phyt. AF228053
Brinjal little leaf phyt. BLL EF186820
Centaurea solstitialis virescence AY270156
Candidatus Phyt. trifolii EY-IL 2 AF409069
Candidatus Phyt. trifoli CP AY390261
Fragaria multicipita phyt. AF190224
Vaccinium myrtillus phytoplasma EU14330
Candidatus Phyt. trifolii CP (VI-A) AY500130
Potato witches-broom phyt. PWB AY500818
Potato witches-broom phyt. DQ256089
Potato witches-broom SK AB076404
Candidatus Phytoplasma trifolii Kr AB279597
Columbia Basin potato purple top phyt. AY692279
Candidatus Phyt. trif. PTL AM260486
Catharanthus phyllody phyt. EF186819
Columbia Basin-potato purple top AY692280
Dry bean phyllody phyt. DBPh3 AY496003
Vinca virescence phyt. AY500817
Iranian cabbage yellows EF592606
Washington potato purple top phyt AY496005
Dry bean phyllody phyt. DBPh2 AY496002
Erigeron witches-broom phyt. AF411592
Argentinian alfalfa witches-broom phyt. AY147038
Candidatus Phyt. fraxi AshY5 AF105316
Candidatus Phyt. fraxini AshY1 AF092209
Candidatus Phyt. fraxini AshY1-#1 AF189215
Paper mulberry witches-broom phit. AY576685
Jujube witches-broom phyt. EF661582
Candidatus Phyt. ziziphi AY072722
Rubus stunt phytoplasma Y16395
Candidatus Phytoplasma ulmi AF122911
Virginia creeper phyt. AF305198
Candidatus Phytoplasma vitis EF581166
Loofah witches-broom phyt. AF086621
Loofah witches-broo phyt. LfWB AF353090
Walnut witches-broom phyt. AF190227
Western X phyt. AF533231
Milkweed yellows phyt. AF510724
Blueberry proliferation phyt. AY034090
Black raspberry witches-broom AF302841
Clover yellow edge phyt. CYE-L AF173558
Clover yellow edge phyt. CYE-C AF175304
Gaillardia phyllody phytoplasma AY049029
Candidatus Phyt. cynodonti BGWL-C1 AJ550984
Candidatus Phyt. cynodonti BGWL-CA AJ550986
Acholeplasma laidlawii M23932
0.02
16SrVI (Clover proliferation group)
16SrVII (Ash yellows group)
16SrV (Elm yellows group)
16SrVIII (Loofah witches'-broom grou
16SrIII (X-disease group)
16SrXIV (Bermuda white leaf group)

Vienna-Collection

In vitro gene bank of fruit tree and grapevine cvs

192 accessions

51 apples

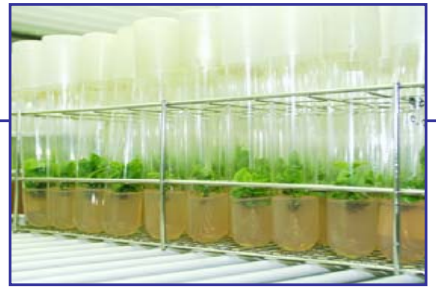
59 plum/cherries

21 apricots/peaches

61 grapevines

In vitro collection of pathogen isolates

114 accessions



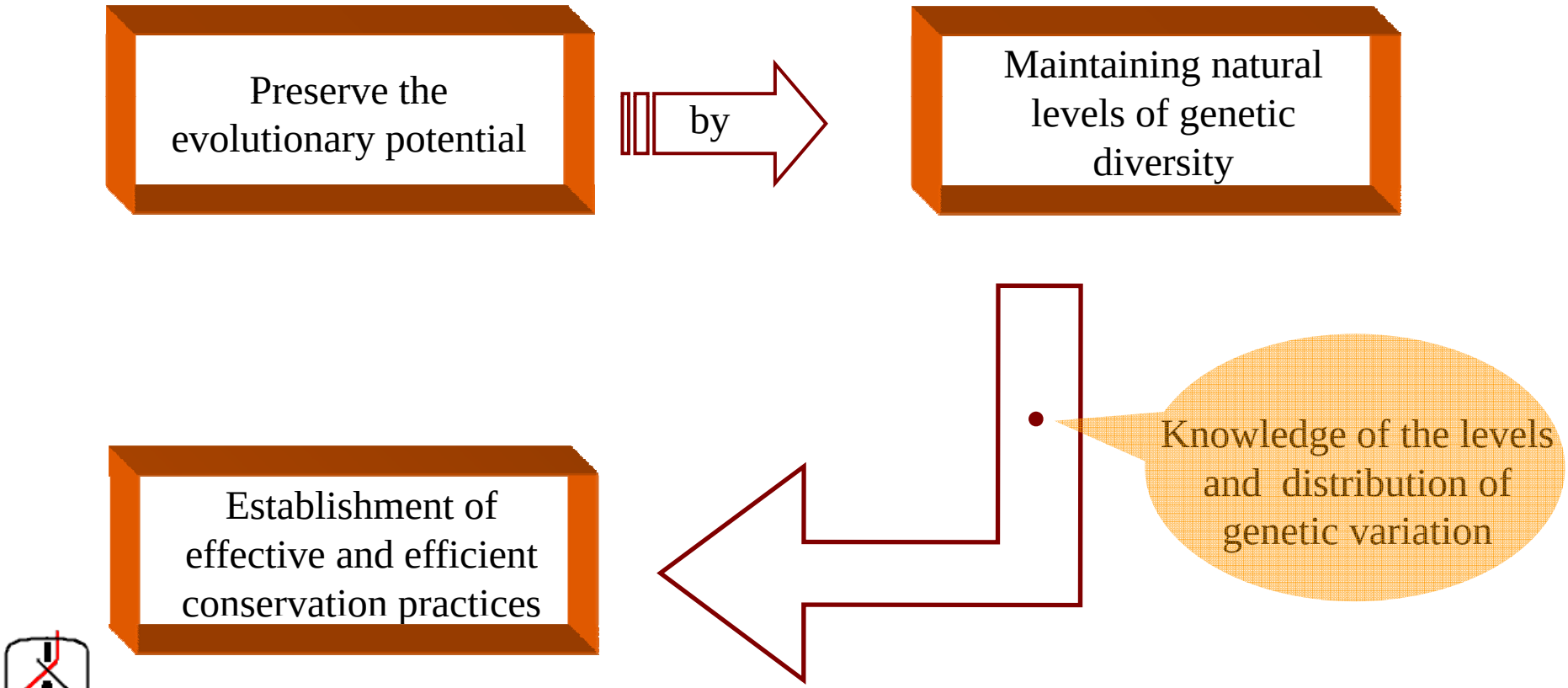
In vivo collection of pathogen-free mother plants

50 accessions

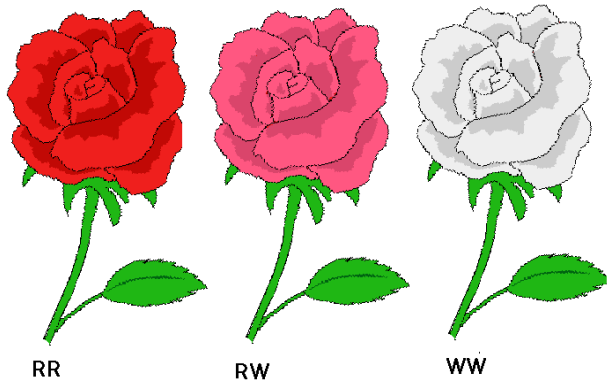
http://www.boku.ac.at/iam/pbiotech/phytopath

Distribution of genetic variation: Implications for conservation

Major goals of conservation genetics



Traditional markers

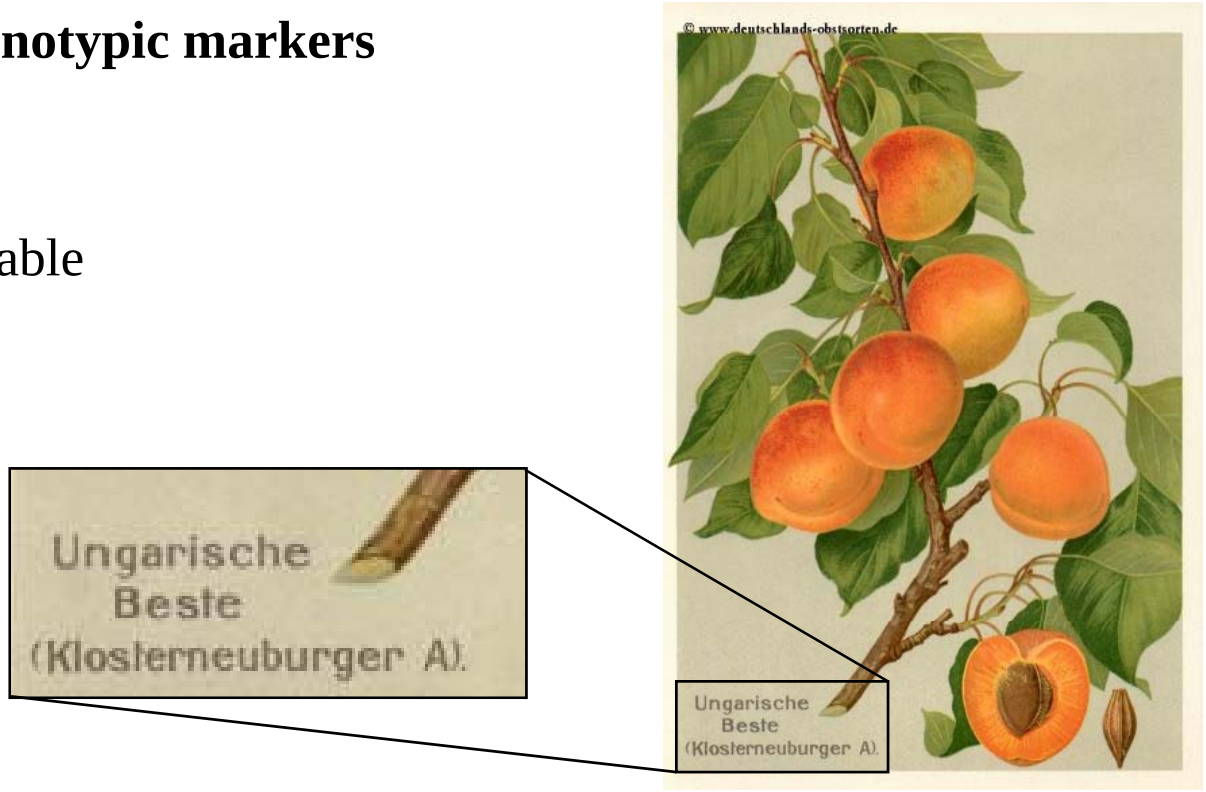


Advantages of phenotypic markers

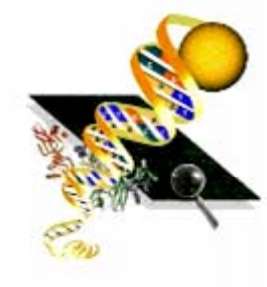
- often easy to score

Disadvantages of phenotypic markers

- low polymorphism
- often multigenic
- environmentally variable



Why molecular markers?



• Allow applications such as:

- tracking of difficult -to-score traits in crosses
- more efficient back-crossing programs
- determination of varietals distinctness and essential derivation
- evaluation of genetic diversity in *ex situ* collections
- studies of *in situ* populations for gene flow, population structure, evolution
- could be used successfully in breeding programmes
-property rights and trade agreements

• Assessments of molecular markers have several advantages:

- Simple inheritance patterns
- Not influenced by environmental factors (selectively neutral)
- Allows precise estimates of genetic diversity

Inheritance of different molecular markers

	Mode of transmission	*Mode of gene action*
Biochemical markers		
Isoenzymes	biparental/nuclear	co-dominant
non-PCR based markers		
RFLP	biparental/nuclear	dominant
Minisatellites	biparental/nuclear	
PCR based markers		
RAPD	biparental/nuclear	dominant
AFLP	biparental/nuclear	dominant
cDNA Marker	biparental/nuclear	co-dominant
Nuclear Microsatellites	biparental/nuclear	co-dominant
SNP	biparental/nuclear	co-dominant
Chloroplast Microsatellites	uniparental	
Mitochondrial marker	uniparental	

Overview of the relevant characteristics of marker technology

	Allozyme	RFLP	Sequencing	RAPD	SSR	AFLP	SNP
Genomic abundance	low	high	Low	high	high	high	high
Level of polymorphism	low	medium	Low	medium	high	medium	high
Locus-specificity	yes	yes	yes	no	yes	no	yes
Co-dominance of alleles	yes	yes	yes	no	yes	no/yes	yes
Reproducibility	high	high	high	low	high	medium/ high	high
Labour intensity	low	high	low/high	low	low	medium	low
Technical demands	low	high	high	low	low/ medium	medium	high
Operational costs	low	high	high	low	low	medium	high
Development cost	low	medium/ high	high	low/ medium	high	low	high
Quantity of DNA require	-	high	low	low	low	medium	low
Amenability to automation	no	no	yes	yes	yes	yes	yes

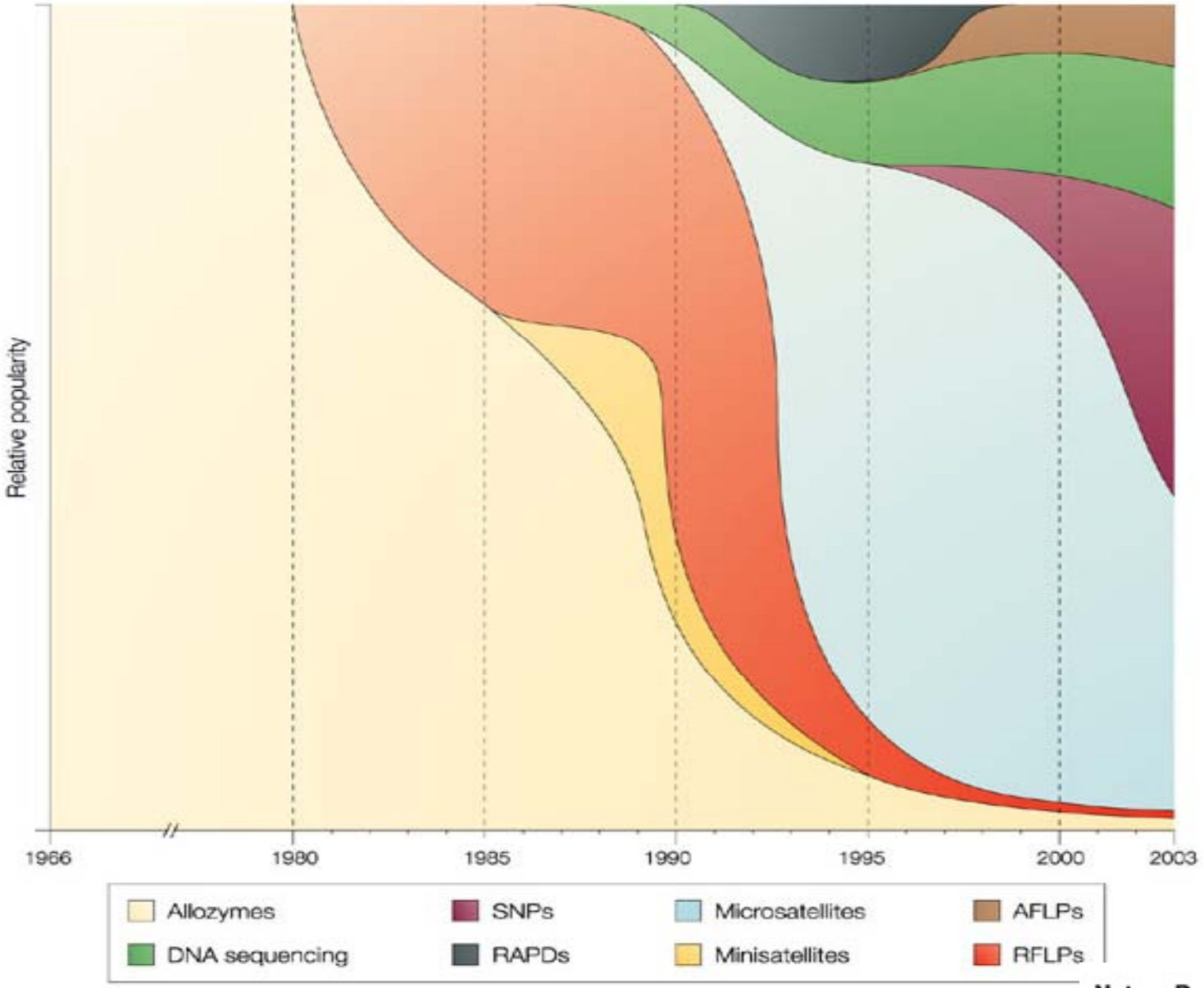
Marker choice:

Which marker for which purpose?

Facts or fashion?

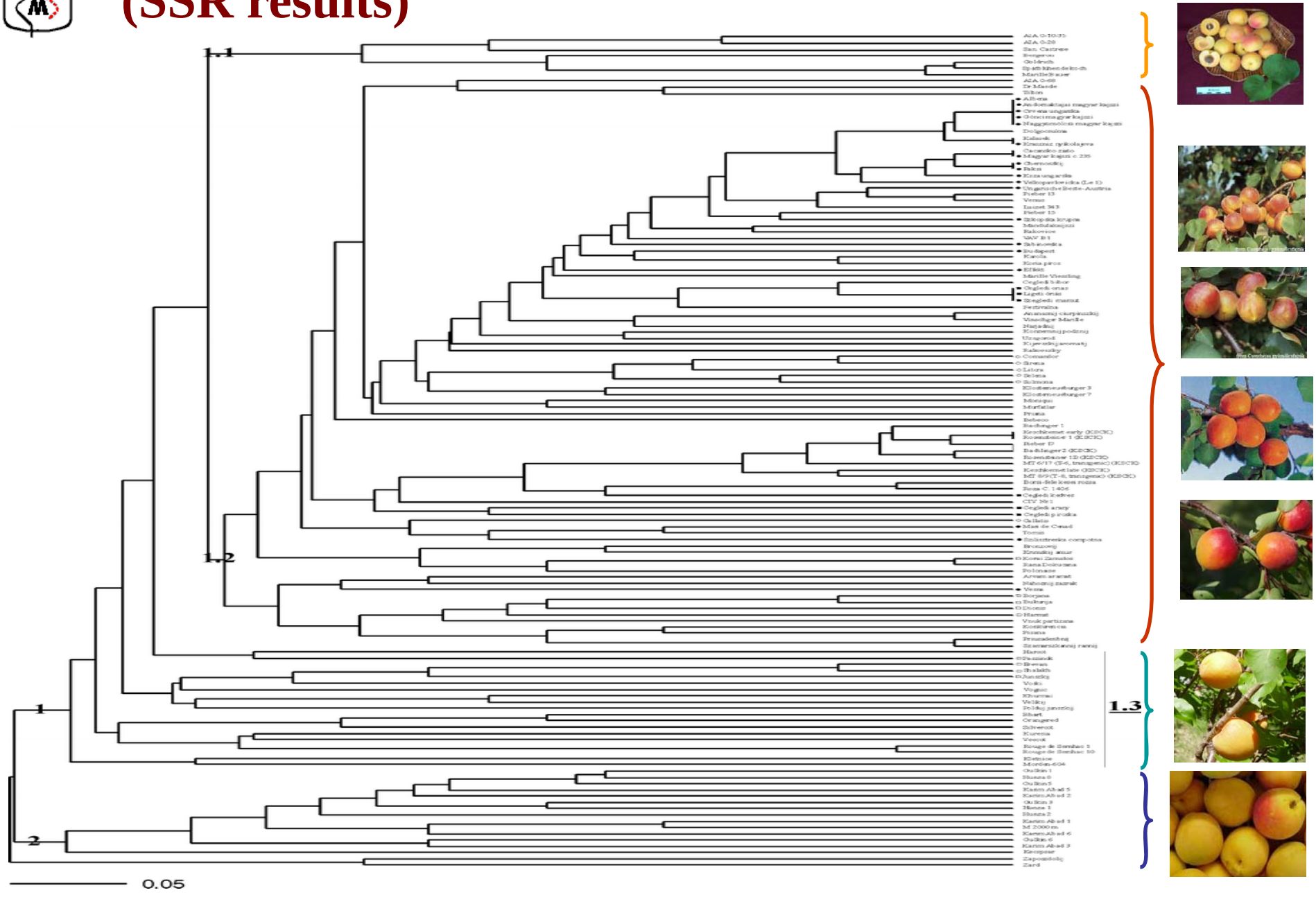
- Which markers will result in the most appropriate levels of discrimination?
- Do results need to be transferred across laboratories?
- How much time (and funding) is available for the project?
- Is sufficient expertise available?
- What are the specific problems inherent to the organism under study?

Subjective view of the changing relative importance of different molecular markers



Nature Reviews | Genetics

UPGMA dendrogram for 133 apricot cultivars (SSR results)



Thank you for your attention