

**Multilocus variable number of tandem repeats  
analysis reveals multiple introductions of  
*Xanthomonas arboricola* pv. *pruni*, causal agent  
of bacterial spot disease of stone fruits and  
almonds, in Spain**

**<sup>1</sup>López-Soriano P, <sup>2</sup>Boyer K, <sup>1</sup>Morente MC, <sup>1</sup>Peñalver J, <sup>2</sup>Grygiel  
P, <sup>3</sup>Palacio-Bielsa A, <sup>2</sup>Vernière C, <sup>1</sup>López MM, <sup>2</sup>Pruvost O.**

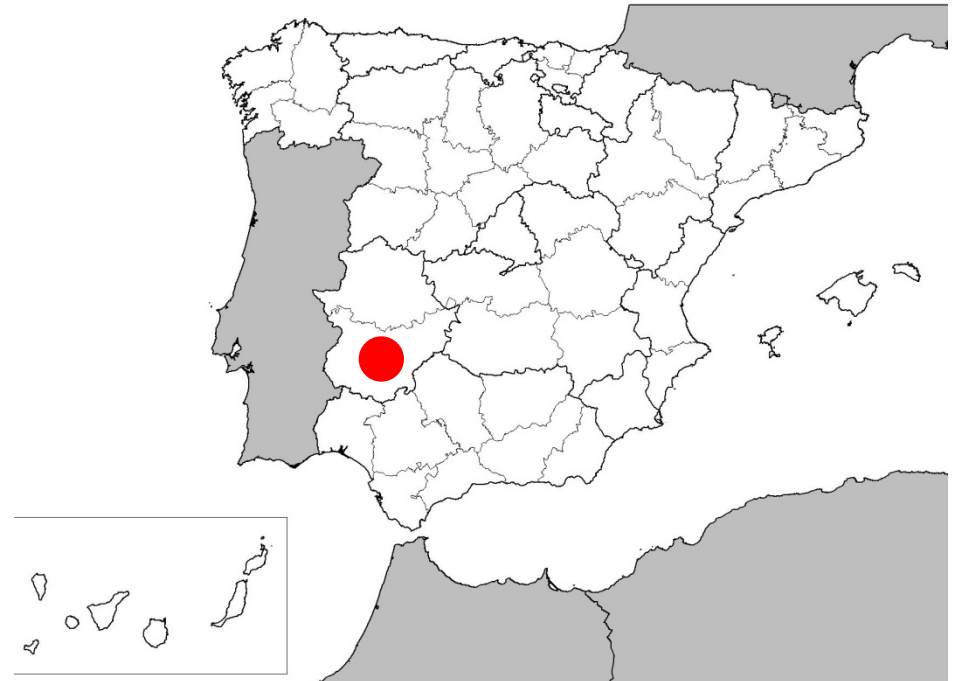
<sup>1</sup>Centro de Protección Vegetal. Instituto Valenciano de Investigaciones Agrarias (IVIA). Moncada, Valencia

<sup>2</sup>UMR Peuplements Végétaux et Bioagresseurs en Milieu Tropical (PVBMT). Saint Pierre, La Réunion (Francia)

<sup>3</sup>Centro de Investigación y Tecnología Agroalimentaria de Aragón (CITA). Montañana, Zaragoza

Xap is listed as a quarantine organism in the EU and the EPPO

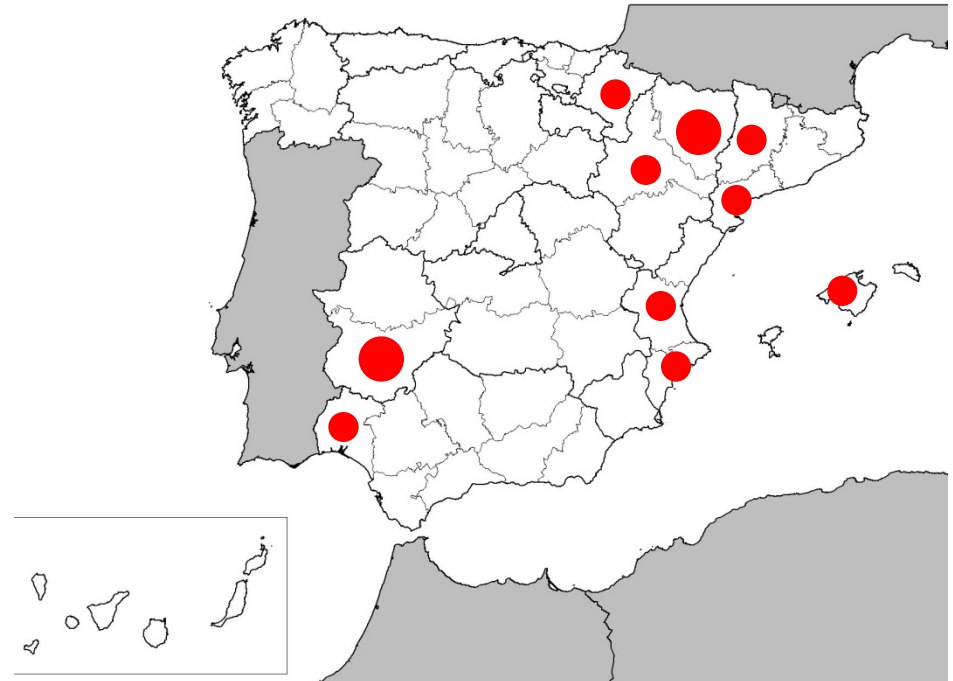
In Spain, it was first detected in the province of Badajoz, in 2002 in  
Japanese plum



Xap is listed as a quarantine organism in the EU and the EPPO

In Spain, it was first detected in the province of Badajoz, in 2002 in  
**Japanese plum**

Since then it has been detected in  
several Spanish provinces and  
different hosts, both in orchards  
and nurseries



- **To assess the genetic diversity of 239 Spanish strains of Xap isolated from 2002 to 2013 in 12 provinces and 25 reference strains from international collections**
- **To establish the epidemiologic relationship between the Spanish strains**

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**Multilocus variable number of tandem repeats analysis (MLVA)**

## MLVA advantages

- Sensitive, rapid and reliable tool
- It shows a high discriminatory power among isolates
- Economic compared to other molecular typing tools
- Amenable to the handling of large samples sets
- Results can be standardized in datasets for comparison among different laboratories

## Xap shows very low genetic diversity among isolates

- That was confirmed in several genetic diversity studies of Xap isolates using different methodologies
  - **FAFLP** (Boudon *et al.*, 2005)
  - **Integron gene cassette arrays and BOX-PCR** (Barionovi & Scortichini, 2008)
  - **Rep-PCR** (Kawaguchi, 2012)
  - **ISSR-PCR** (Kawaguchi, 2014)

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- Higher resolution obtained with MLVA
  - Genetic diversity study of Xap isolates of *Prunus laurocerasus* from the Netherlands (Bergsma-Vlami *et al.*, 2012)
  - Genetic diversity study of Xap isolates and other pathovars of *X. arboricola* (Cesbron *et al.*, 2014)



## MLVA

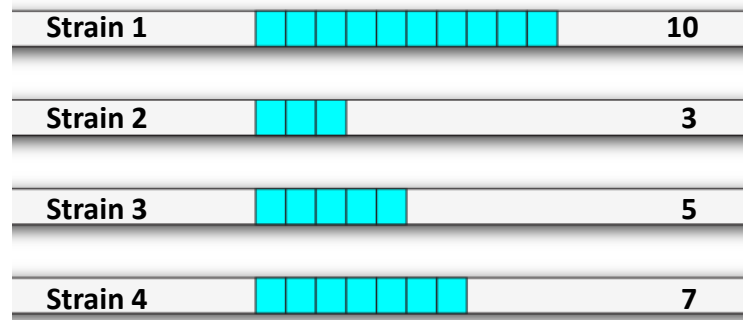
- It is based in the natural variation in the number of tandem repeated (TR) DNA sequences found in the microbial genome of most bacterial species

GGATAGTATTC **GAATCGG** **GAATCGG** **GAATCGG** **GAATCGG** CCGA

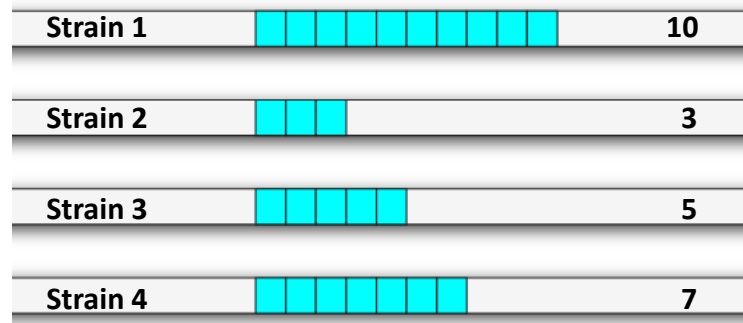
Microsatellites: less than 10 nucleotids

Minisatellites: 10-60 nucleotids

- The number of TRs in a particular locus may differ between different strains

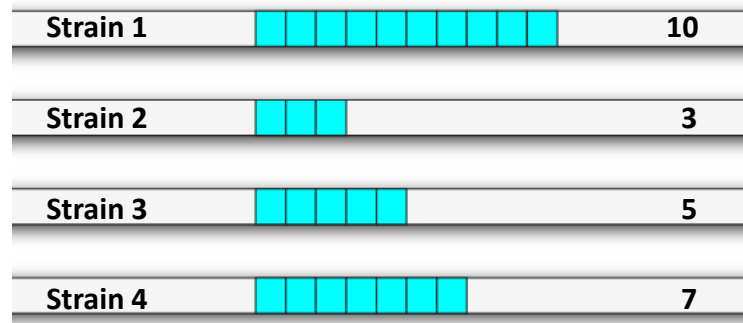


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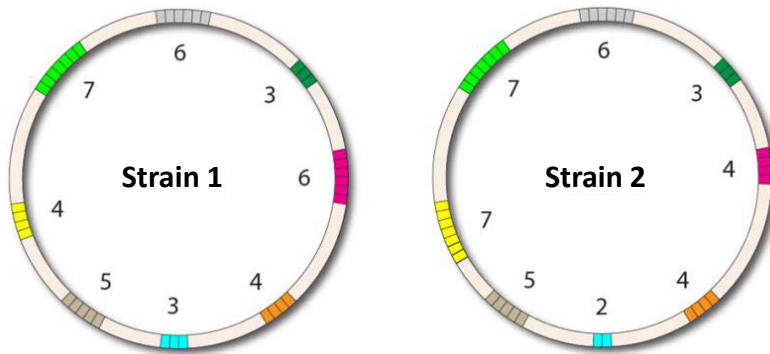


- Because of this variation such loci are designated as **variable number of tandem repeat (VNTR) loci**

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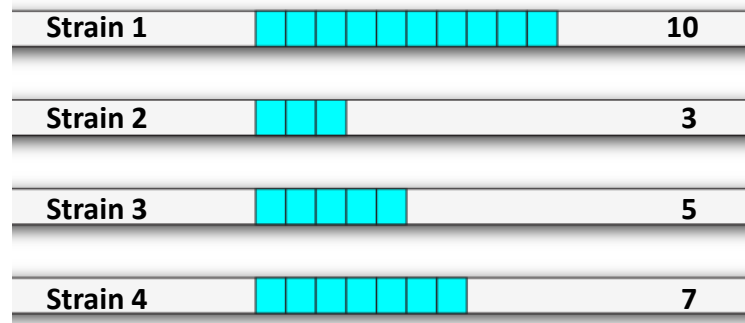
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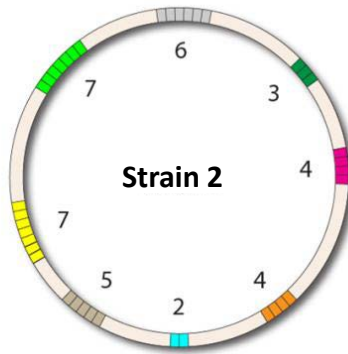
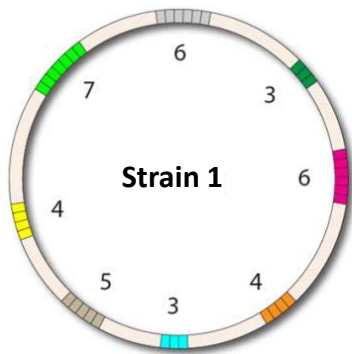
MLVA profile strain 1: 6-3-6-4-3-5-4-7

MLVA profile strain 2: 6-3-4-4-2-5-7-7

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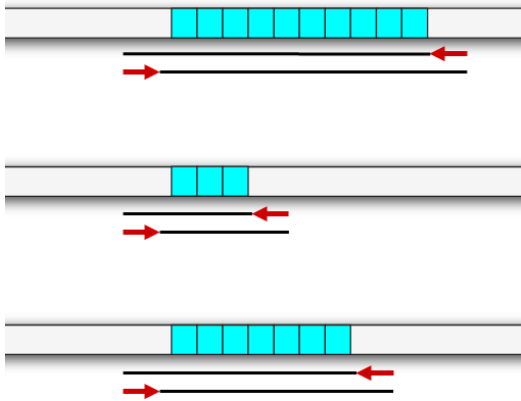
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MLVA profile strain 1: 6-3-6-4-3-5-4-7

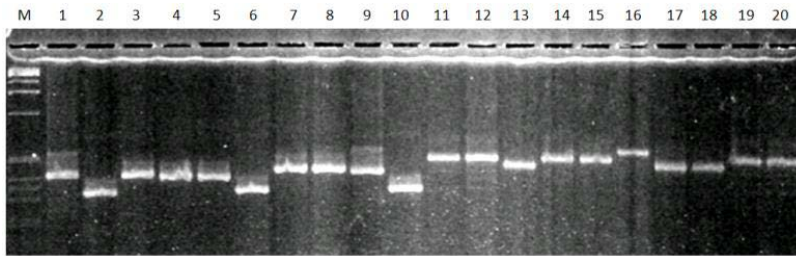
MLVA profile strain 2: 6-3-4-4-2-5-7-7

- The variation in the numbers of repeats in a set of VNTR loci is achieved by PCR

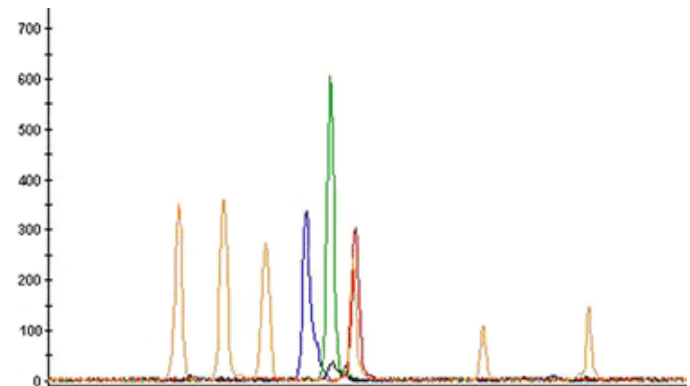


Primers target the conserved flanking regions of the tandem repeats

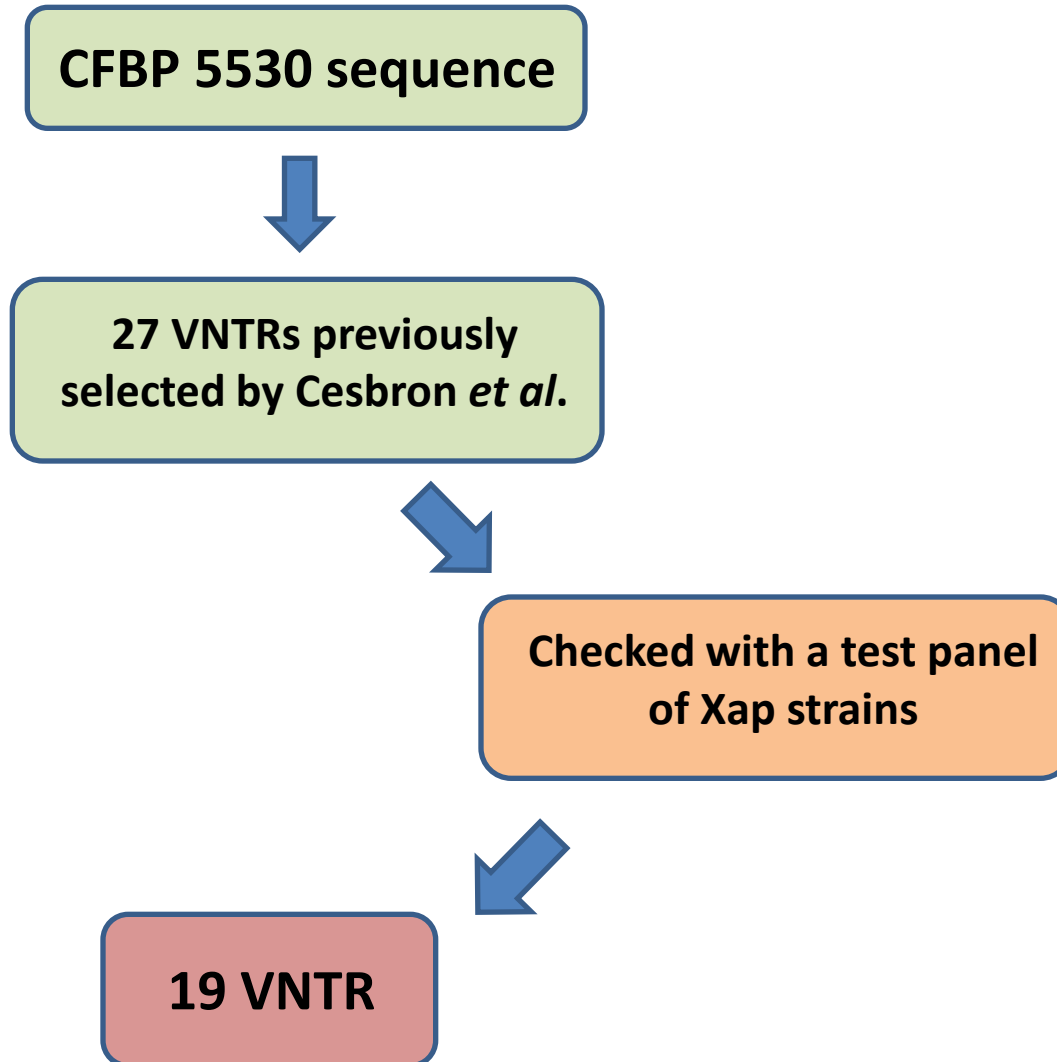
- PCR products are revealed by

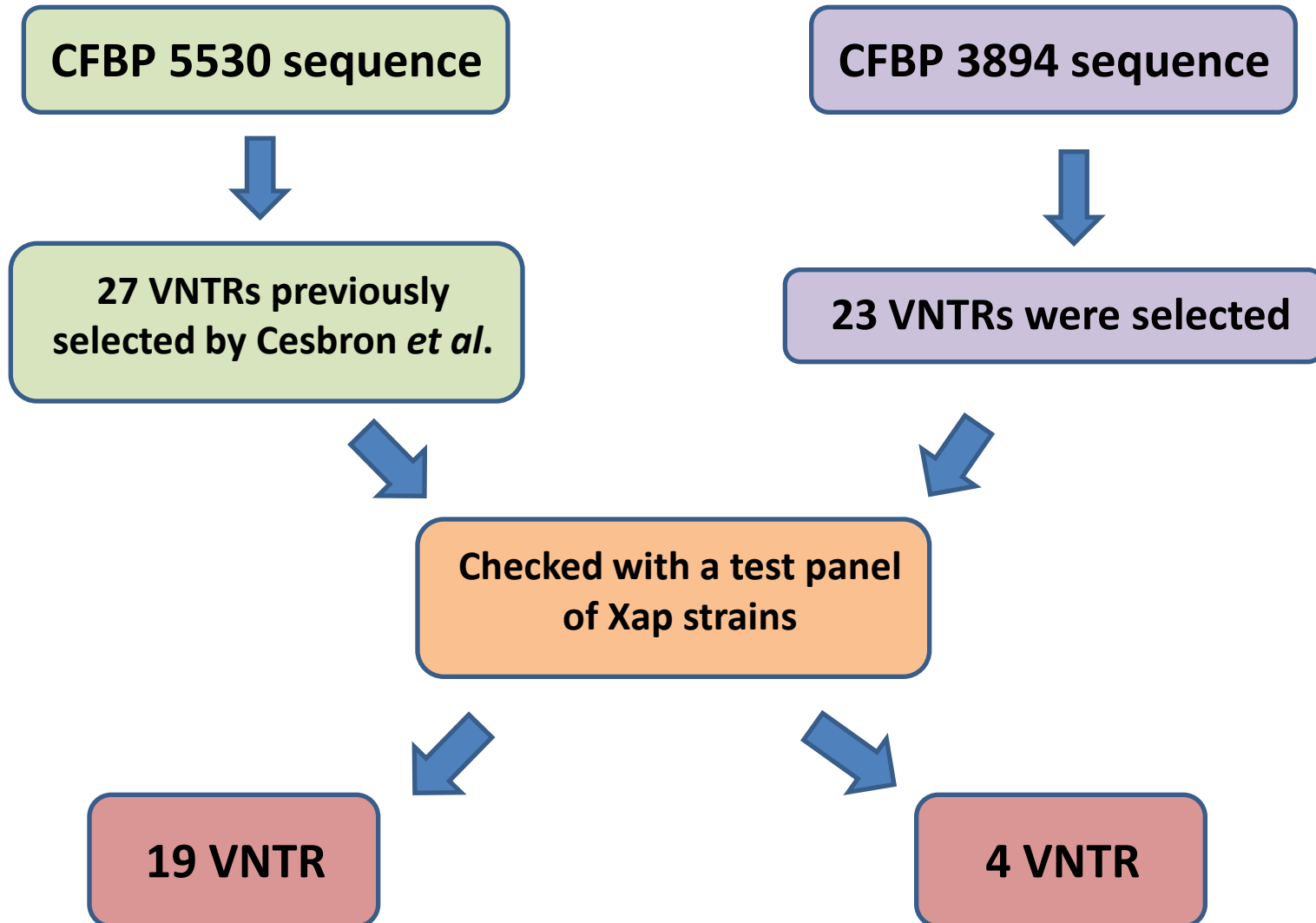


**Agarose gels**



**Capillary electrophoresis**







Microsatellites			
TR 51I	TR 37I	TR 37II	TR 05-06
TR 40I	TR 36I	TR 03I	TR 68I
TR 30II	TR 28II	TR 38II	TR 58II
TR 33I	TR 58I	TR 67II	TR 79I
TR 50I	TR 66I	TR 15I	

Minisatellites
Xap 4790
Xap 0897
Xap 2280
Xap 2244
TR 10II

## Multiplex-PCR

- ✓ VNTRs grouped according to their annealing temperature
- ✓ Primers labeled with fluorescent dyes (FAM, PET, NED, VIC)
- ✓ PCR of 264 strains

Capillary electrophoresis

Electrophoregrams analysis

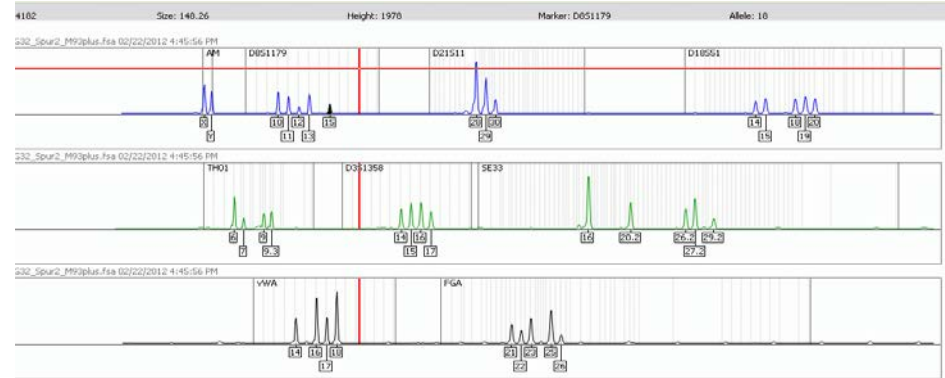
(GeneMapper 4.0, Applied Biosystems)



Allelic profiles of 255 strains



142 Haplotypes



Cepa	TR5 1I	TR3 7I	TR05- 06	TR37I I	TR40 I	TR3 6I	TR6 8I	TR03 I	TR30 II	TR28 II	TR58 II	TR38 II	TR3 3I	TR5 8I	TR7 9I	TR3 9II	TR7 6I	TR5 0I	TR1 5I	TR66 I	TR18 II	TR67I I	Xap47 90	Xap44 22	Xap08 97	Xap22 80	Xap25 15
2626.1	5	4	16	9	8	5	9	7	8	4	11	8	6	7	7	20	6	5	7	10	8	14	4	3	3	13	6
2626.3	5	5	17	9	8	5	13	7	7	4	14	9	6	6	7	23	6	8	6	11	17	13	4	3	3	8	6
2835.4	5	5	19	9	8	5	13	7	7	4	14	9	6	6	7	17	6	7	6	10	16	12	4	3	3	8	6
3162.1	5	4	17	9	7	5	11	7	10	4	12	9	5	6	7	21	6	6	7	11	21	15	4	3	3	12	5
3604.10	6	5	16	9	8	5	14	7	7	4	15	10	5	6	7	23	6	7	6	10	16	19	4	3	3	8	6
3767.1	5	6	18	9	8	5	15	7	7	4	14	9	6	6	6	23	6	6	6	9	13	14	2	3	3	7	7
4330	5	5	20	9	8	5	13	7	7	4	14	9	6	6	7	17	6	8	6	9	13	12	4	3	3	8	5
CFBP5530	5	5	19	9	8	5	13	7	7	4	14	10	7	6	7	17	6	7	6	9	17	14	4	3	3	8	6
DAR41285	4	4	20	9	8	5	13	8	8	4	13	10	7	5	7	17	6	5	7	9	16	5	4	2	3	11	5

Capillary electrophoresis

Electrophoregrams analysis

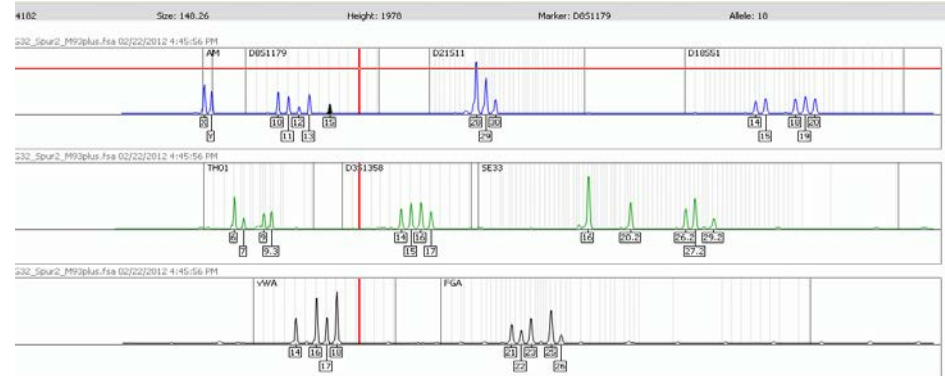
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Capillary electrophoresis

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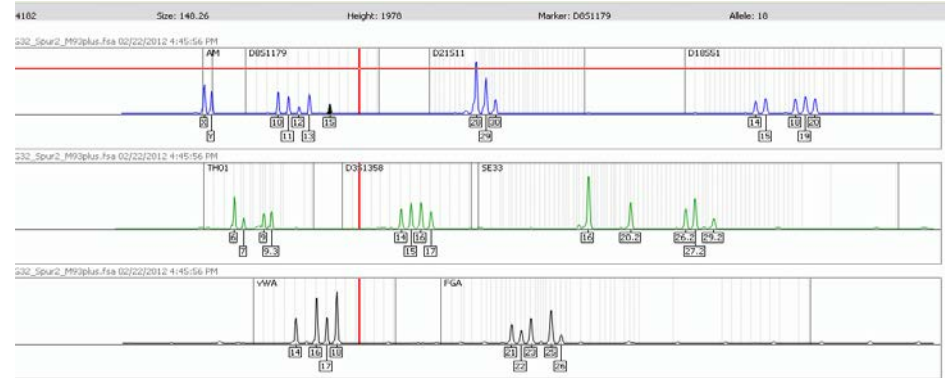
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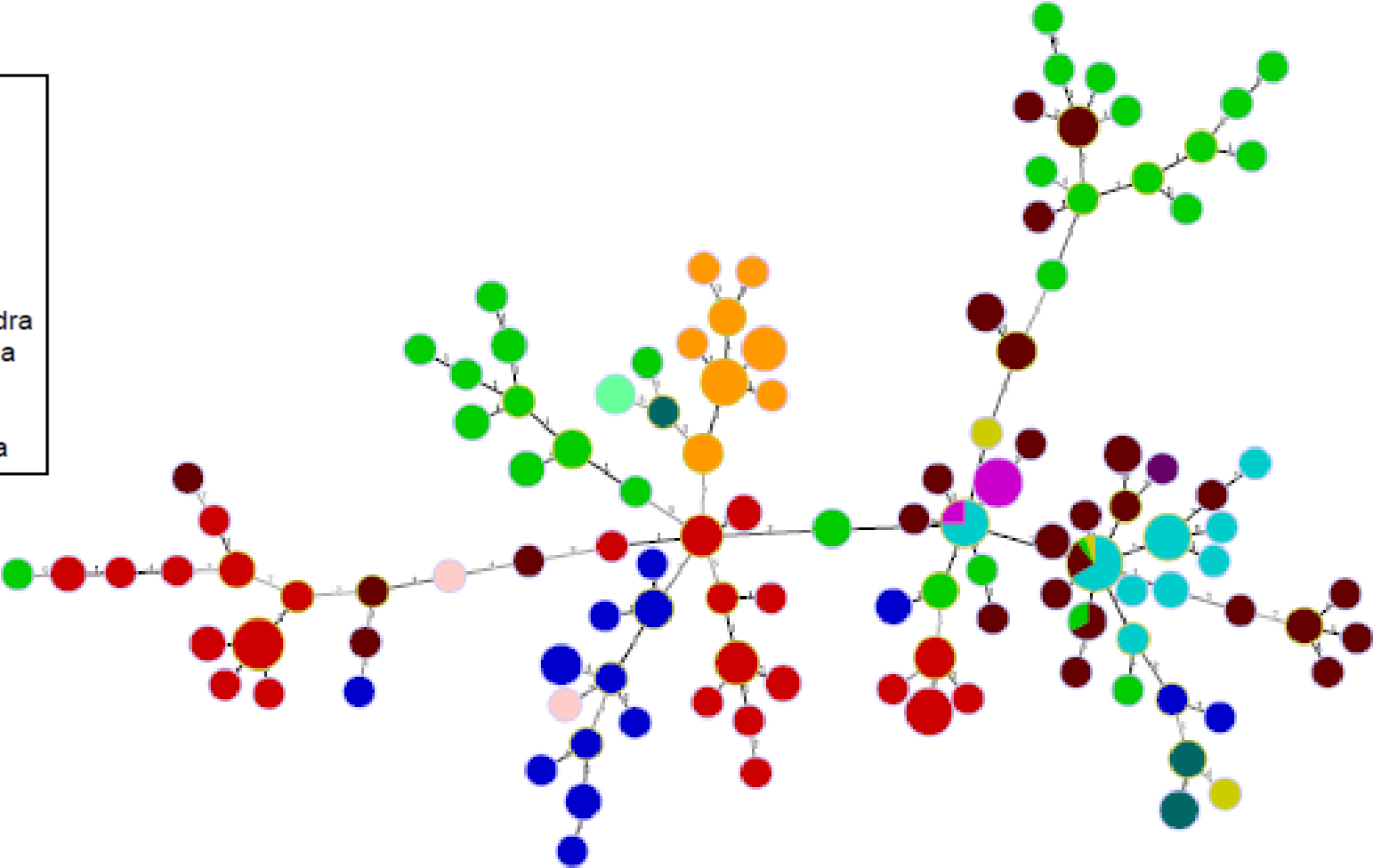
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# Minimum Spanning Tree (MST) of Spanish strains

- Alicante
- Badajoz
- Huelva
- Huesca
- Lleida
- Mallorca
- Navarra
- Pontevedra
- Tarragona
- Teruel
- Valencia
- Zaragoza



Restriction of 4 TR  
loci variation

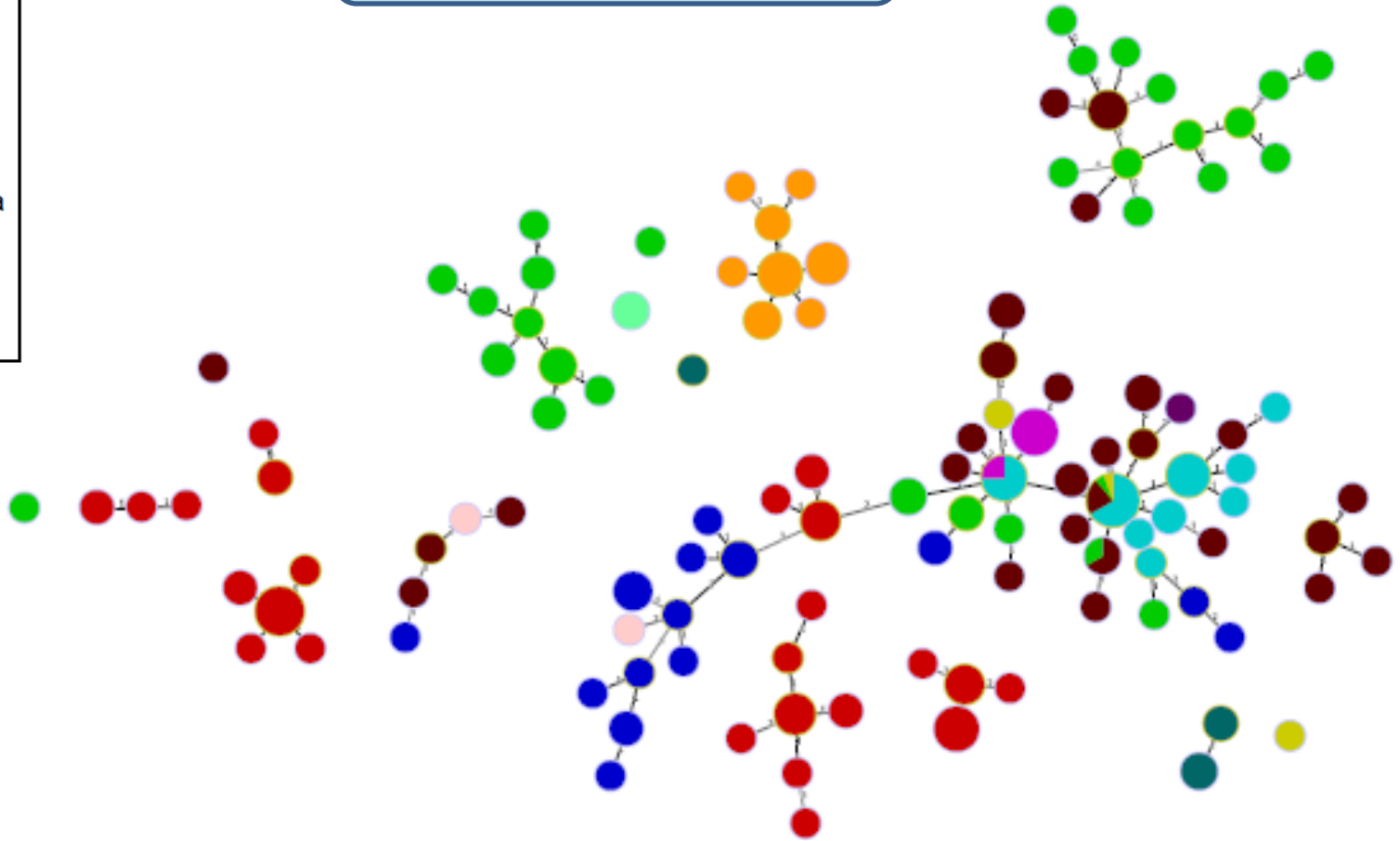


18 genetic clusters



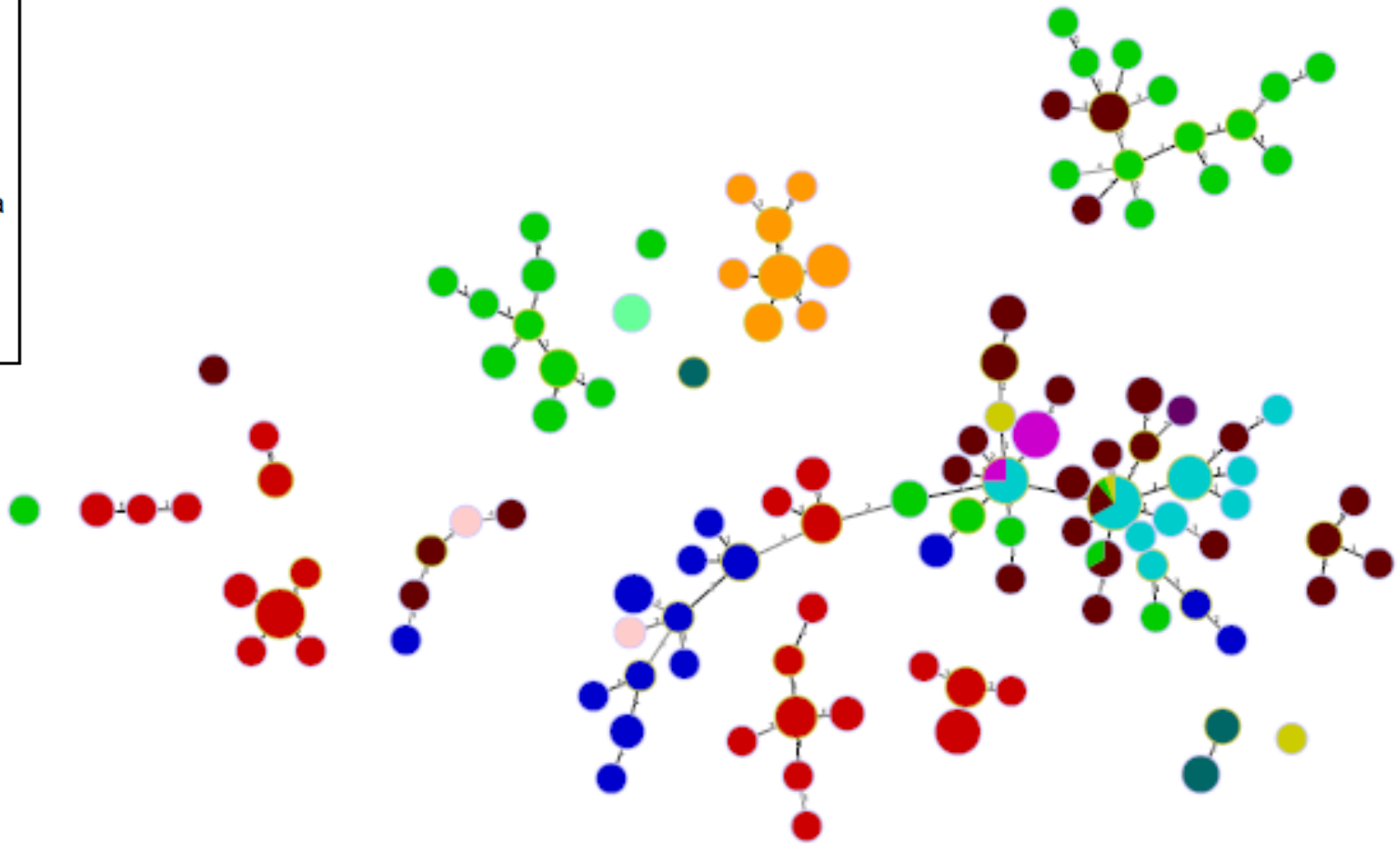
Multiple introductions

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## Nurseries play an important role in the introduction

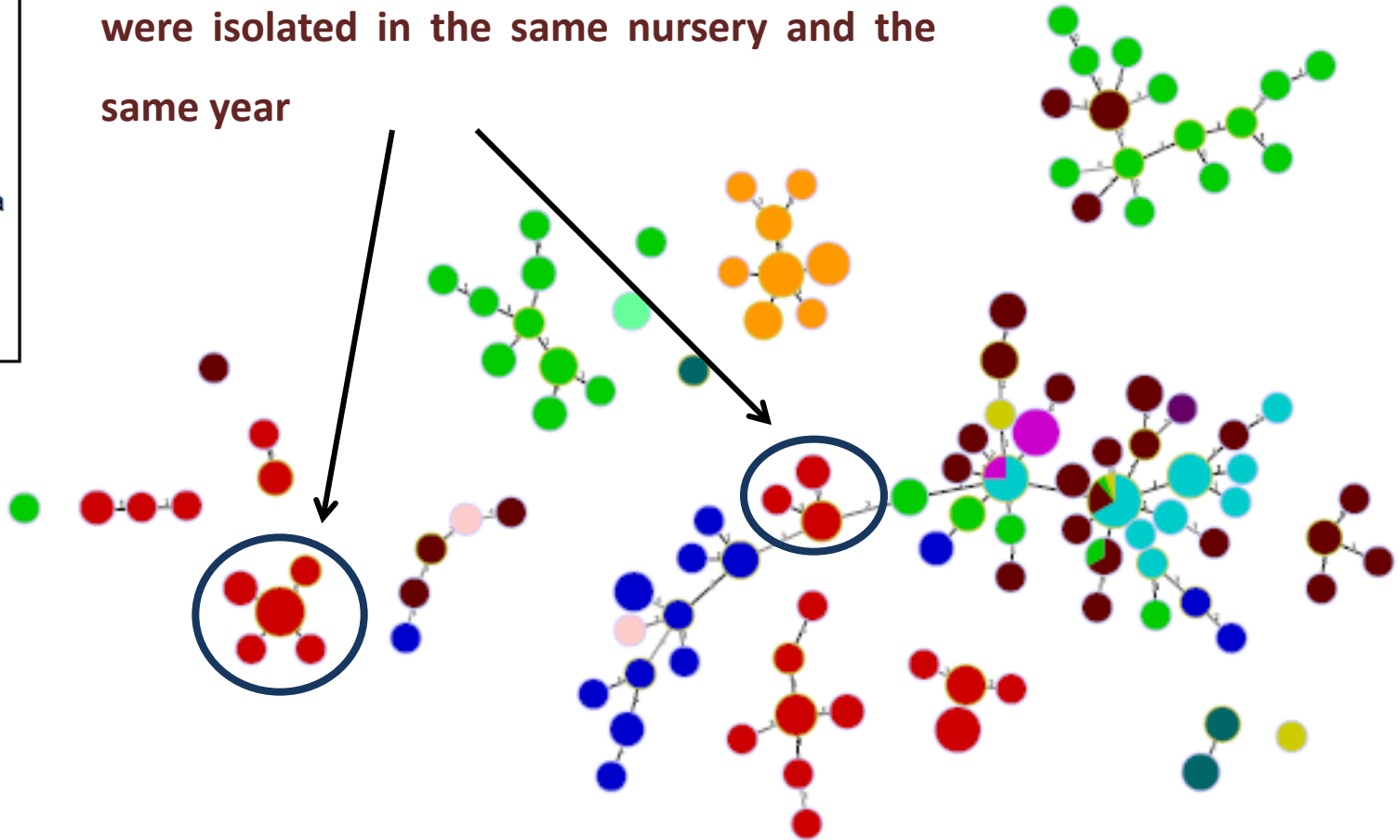
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## Nurseries play an important role in the introduction

Strains showing very different allelic profiles were isolated in the same nursery and the same year

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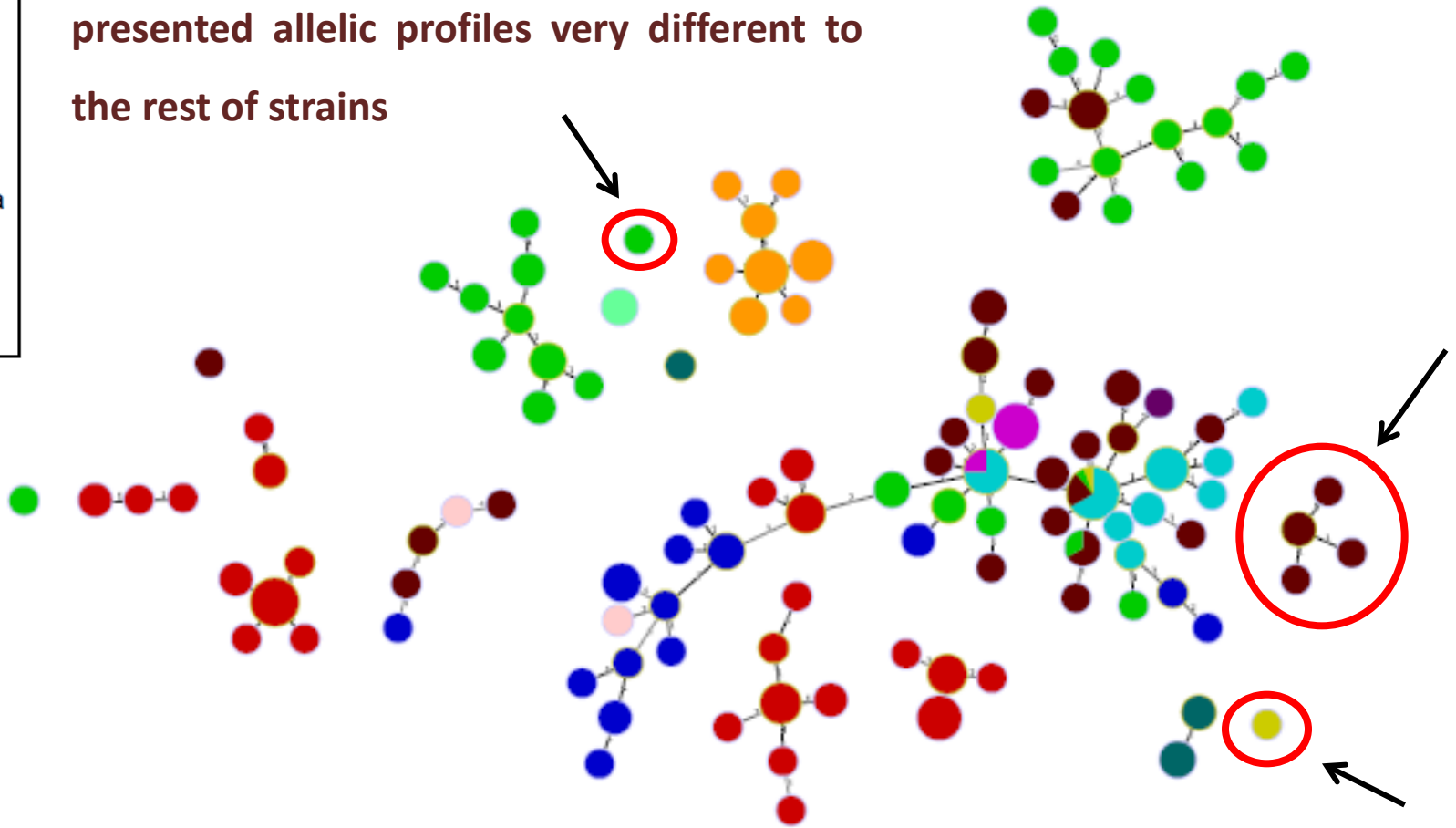




# Nurseries play an important role in the introduction

Strains isolated in other nurseries presented allelic profiles very different to the rest of strains

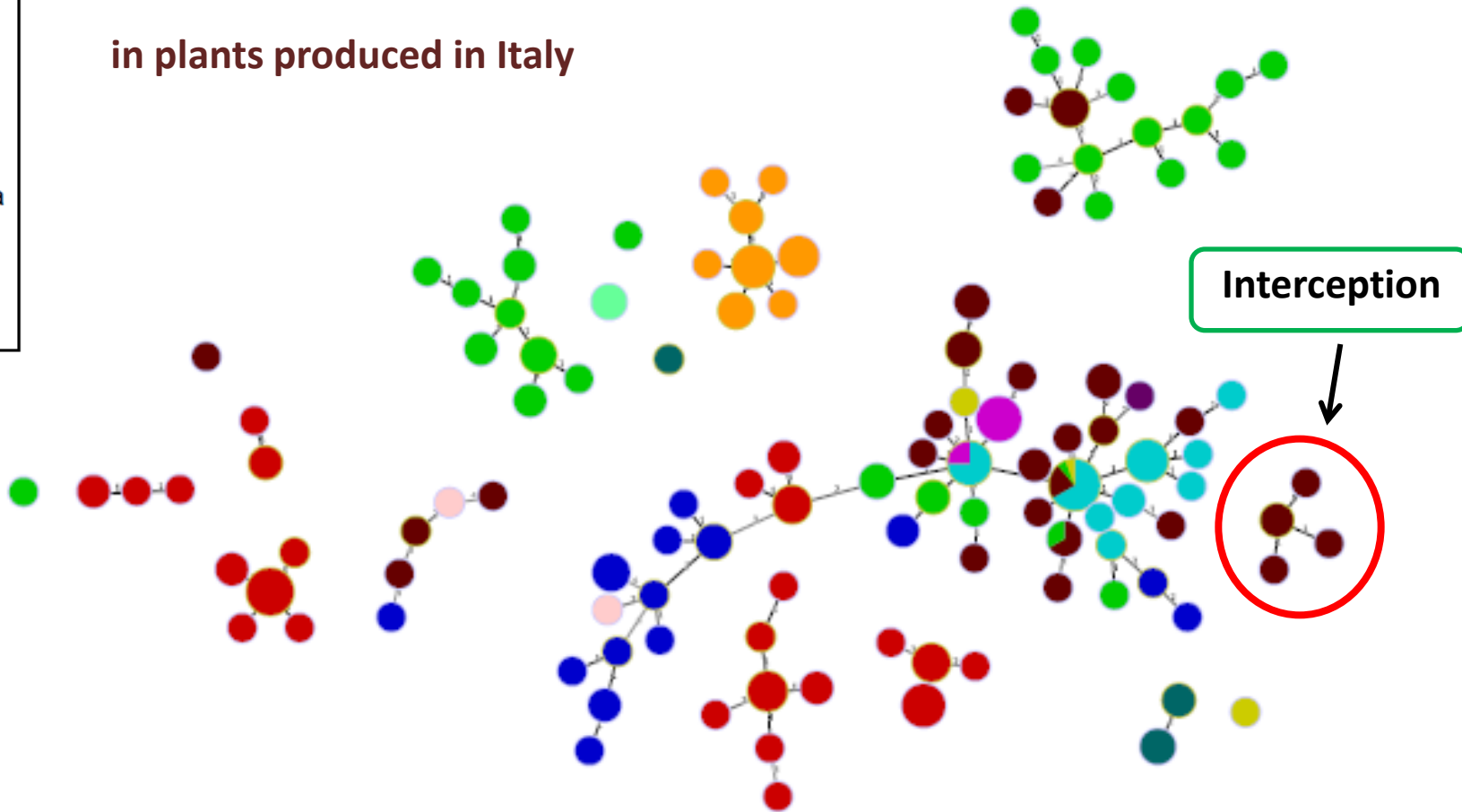
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## Nurseries play an important role in the introduction

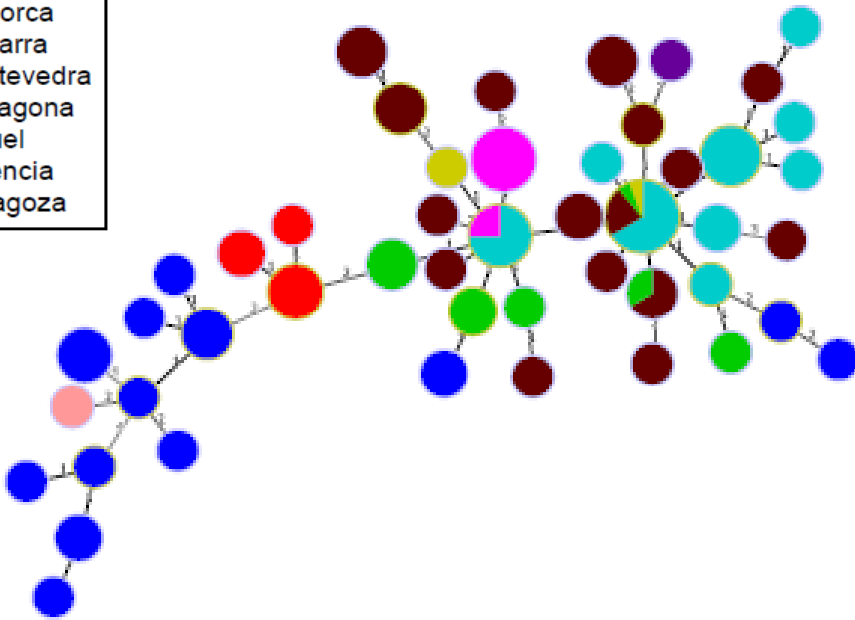
4 strains isolated in a Spanish nursery but  
in plants produced in Italy

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## Dissemination of Xap through the Spanish nurseries

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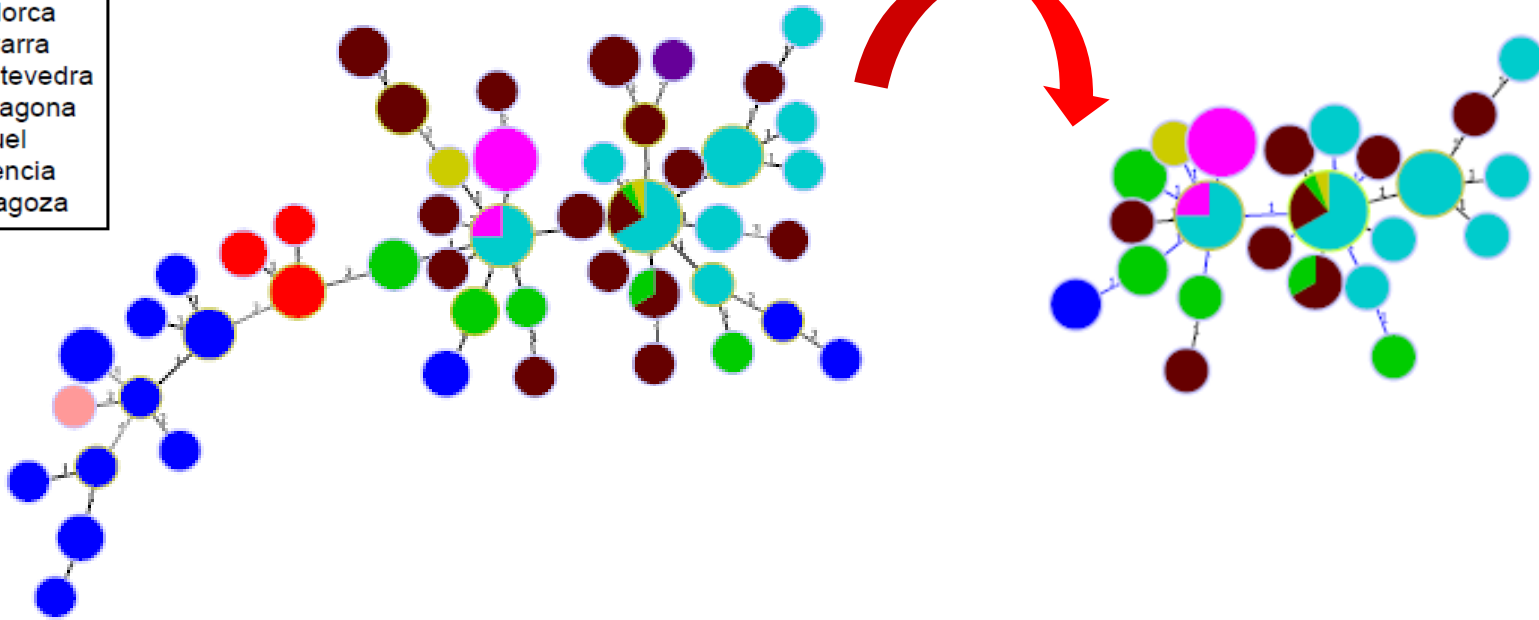
**Genetic cluster**: haplotypes less than 4 loci variants

**Related strains**

**Almost 50% of total collection was grouped here**

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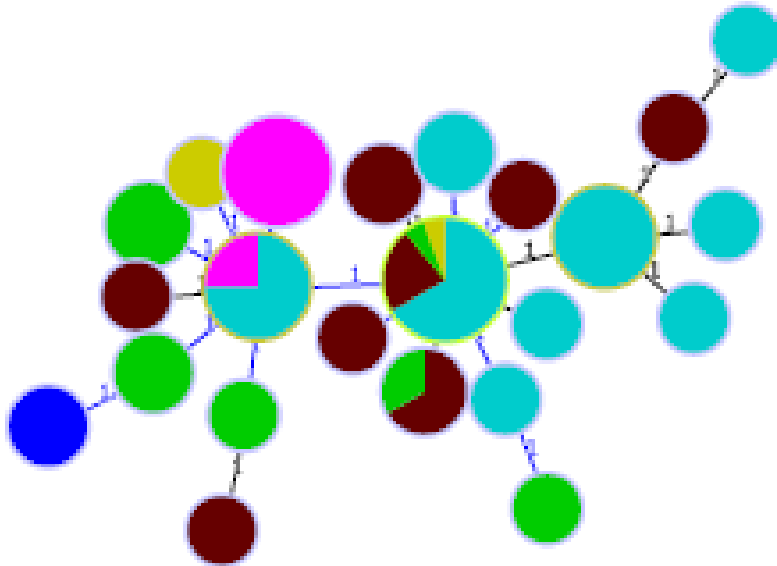
**Almost 50% of total collection was grouped here**

**Clonal complex**: haplotypes single locus variants

**Closely related strains**

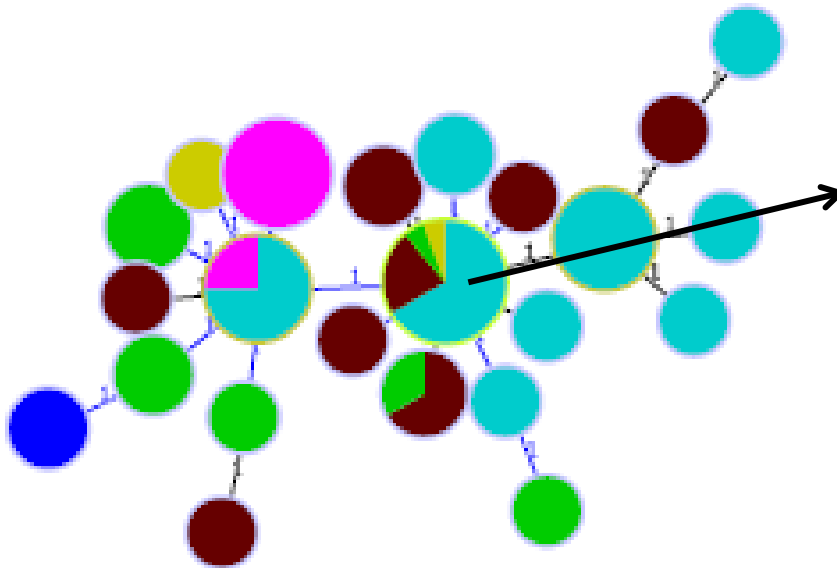
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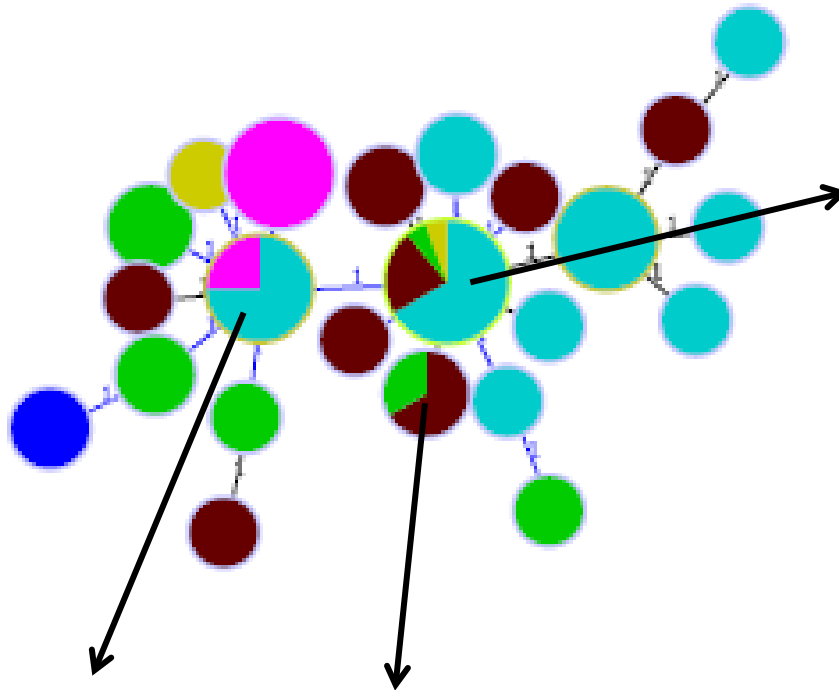
Strains from 4 provinces, including 2 nurseries from 2 different provinces



Exchange of contaminated plant material between nurseries

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Strains from 4 provinces, including 2 nurseries from 2 different provinces



Exchange of contaminated plant material between nurseries

Haplotypes including strains isolated in nurseries and orchards located in different provinces



Transmission from nurseries to orchards

- **Multilocus Variable Number of Tandem Repeats Analysis is an efficient tool to assess the genetic diversity of Xap strains**
- **Our MLVA scheme allowed us to identify 142 haplotypes in a collection of 264 strains**
- **Results obtained reveal multiple introductions of Xap in Spain especially by trade of contaminated plant material from foreign nurseries**
- **MLVA scheme confirms dissemination of the pathogen through Spanish nurseries**





**Thank you for your  
attention**