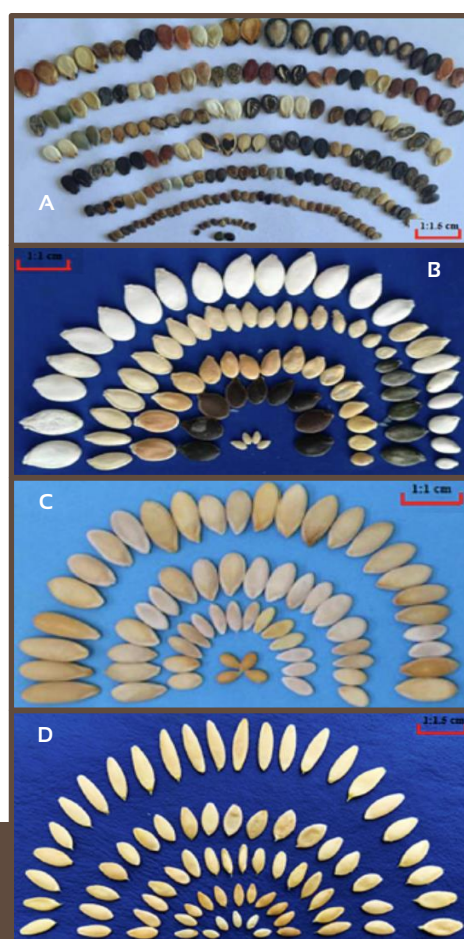


## *Pseudomonas syringae*-induced diseases in cucurbits

### ➤ Cucurbits:

- Among the **most cultivated crops**
- Most **economically important** species: watermelon, melon, cucumber...
- Remarkable **diversity** in seeds and in fruit size, shape and colour

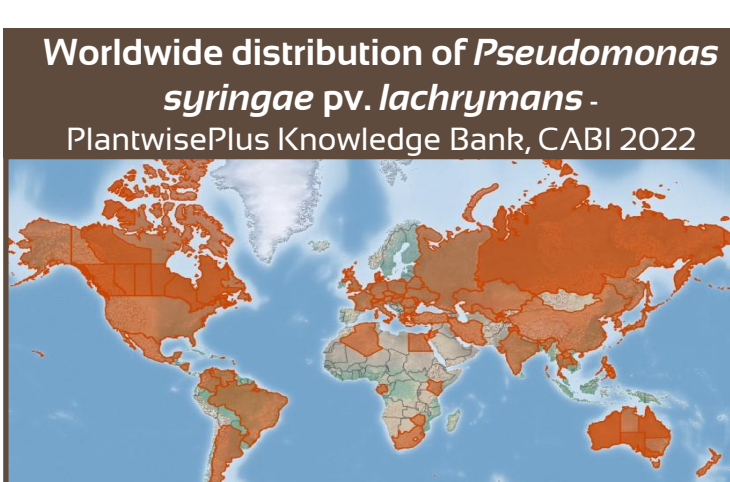
Seed size variation among cucurbit crops  
A: watermelon, B: Pumpkin/squash, C: Melon, D: Cucumber  
Guo et al., 2020



### ➤ Examples of seed-borne diseases:

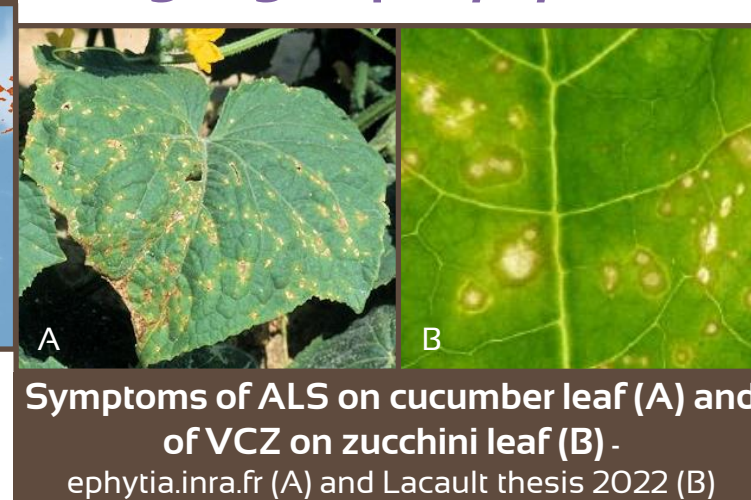
#### ➤ Angular leaf spot (ALS) caused by *P. syringae* pv. *lachrymans*:

- Most widespread** bacterial disease of all cucurbit crops
- Significant yield reduction** in the number of fruits and fruit weight
- Symptoms:** Angular spots with yellow halo on leaves and water-soaked tan, small circular spots on fruits



#### ➤ Vein Clearing on Zucchini (VCZ) caused by lineages of *P. syringae*:

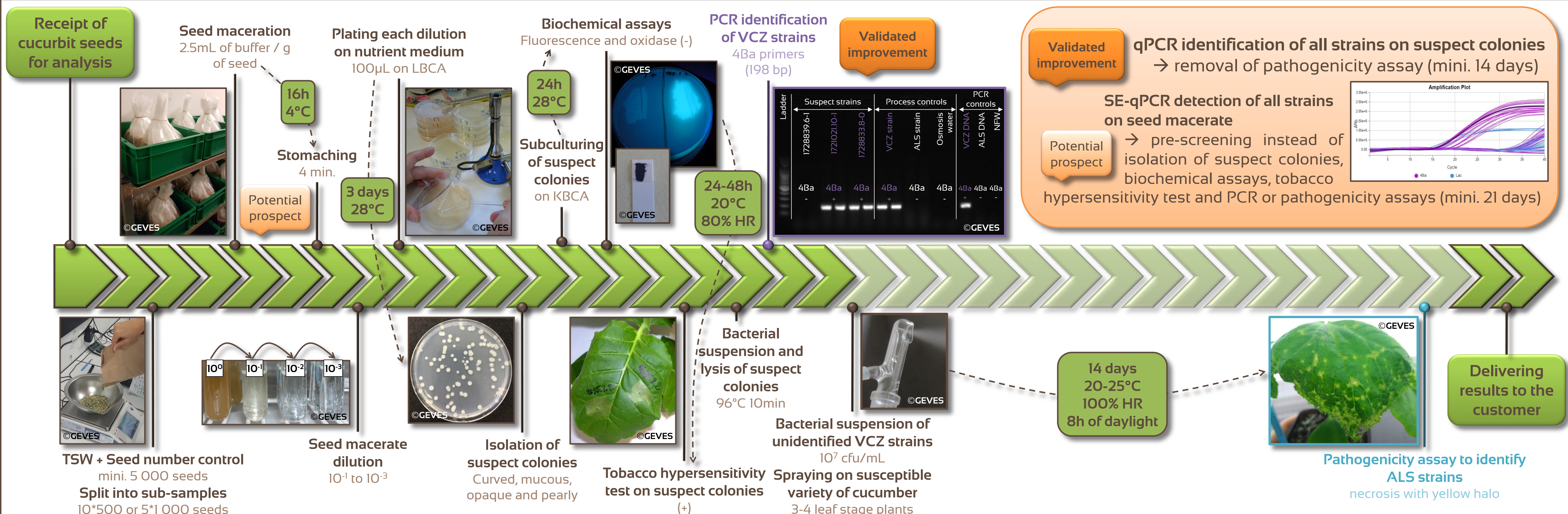
- First observation: **2004**
- Causal agent was provisionally named *P. syringae* pv. '*peponis*'



### ➤ Diseases management:

- Resistant crop cultivars
- Cultural practices
- Chemical and biological control
- Seed health analysis**
  - Bacteriological analysis of cucurbit seeds at **GEVES** (detection and identification of **ALS** and **VCZ** strains)
  - Around **450 analyses per year** (approx. 20%)
  - Analysis performed on **5 000 seeds** and in **36 days**

## Cucurbit seeds detection of *Pseudomonas syringae* strains responsible of diseases



## Development of a qPCR identification method for *Pseudomonas syringae*-induced diseases in cucurbits

### 1. Characterisation of the strain collection:

- Host and morphological** criteria
- Biochemical and tobacco hypersensitivity** tests
- Identification PCR
  - PCR 4Ba** for VCZ strains (Manceau et al., 2011)
  - other PCRs for non-target strains
- Pathogenicity assays** (\*result from provider)
  - on zucchini or cucumber for target
  - according to bacterial species for non-target
- Phylogenetic analyses
  - Multilocus sequence analysis** based on concatenated partial sequences of *cts*, *gapA*, *gyrB* and *rpoD* (Berge et al., 2014)
  - PCRs and qPCRs to specifically identify **2ba** and **2a** phylogenetic clusters and to confirm **2b** and **2d** phylogroups (Lacault et al., 2023)

**5 ALS strains**  
+ **24 VCZ strains** (15 alongside)  
+ **12 *P. syringae* pathogenic strains** (10 alongside)  
**41 target strains**

**33 non-target strains** (20 alongside)  
+ **3 *P. syringae* non-pathogenic strains**  
**36 non-target strains**

### 2. ALS specificity and sensitivity:

- Lac primers/probe** designed by GEVES
- Amplification of **5 out of 5 ALS strains**
- Amplification of **1 strain out of 7 of '*maculicola*'**
- No-amplification of the **24 VCZ strains**, the **12 *P. syringae* pathogenic**, the **3 *P. syringae* non-pathogenic** and the **32 other non-target**

|            | Expected + | Expected -            |
|------------|------------|-----------------------|
| Obtained + | 5          | 1                     |
| Obtained - | 0          | 24 + 12 + 3 + 32 = 71 |

Specificity = **98.61 %**

Sensitivity = **100 %**

| Strains                                                                | Name                          | Host      | Country     | Date | Provider  | PCR 4Ba | Pathogenicity assay    | Phylo-group | qPCR 4Ba | Lac |
|------------------------------------------------------------------------|-------------------------------|-----------|-------------|------|-----------|---------|------------------------|-------------|----------|-----|
| ALS target strains                                                     | CFBP2104                      | cucumber  | USA         | 1935 | CFBP      | -       | + on cucumber          | 1a          | -        | +   |
|                                                                        | CFBP8534                      | cucumber  | USA         | 1935 | CFBP      | -       | + on cucumber          | 1a          | -        | +   |
|                                                                        | CFBP6463                      | cucumber  | Hungary     | 1958 | CFBP      | -       | + on cucumber          | 3           | -        | +   |
|                                                                        | psLO6-1                       | na        | na          | na   | NSHS      | -       | + on cucumber          | 3           | -        | +   |
|                                                                        | Ps17                          | na        | na          | na   | Syngenta  | -       | na                     | na          | -        | +   |
|                                                                        | Ps pep 99                     | zucchini  | na          | na   | HM.Clause | +       | + on zucchini          | 2ba-A       | +        | -   |
|                                                                        | P12855                        | zucchini  | Chile       | 2007 | HM.Clause | +       | + on zucchini          | 2ba-A       | +        | -   |
|                                                                        | P137                          | zucchini  | France      | 2014 | HM.Clause | +       | + on zucchini          | 2ba-A       | +        | -   |
|                                                                        | Peponis9                      | na        | na          | na   | Syngenta  | +       | na                     | 2ba-A       | +        | -   |
|                                                                        | P1                            | zucchini  | France      | 2005 | HM.Clause | +       | + on zucchini          | 2ba-B       | +        | -   |
| VCZ target strains                                                     | P69                           | zucchini  | USA         | 2010 | HM.Clause | +       | + on zucchini          | 2ba-B       | +        | -   |
|                                                                        | P88                           | zucchini  | China       | 2011 | HM.Clause | +       | + on zucchini          | 2ba-C       | +        | -   |
|                                                                        | P100                          | zucchini  | na          | 2011 | HM.Clause | +       | + on zucchini          | 2ba-C       | +        | -   |
|                                                                        | ZUM3584                       | na        | na          | 2023 | Syngenta  | +       | na                     | 2ba-C       | +        | -   |
|                                                                        | P77                           | zucchini  | France      | 2010 | HM.Clause | +       | + on zucchini          | 2a-D        | +        | -   |
|                                                                        | P70                           | zucchini  | Thailand    | 2010 | HM.Clause | +       | + on zucchini          | 2a-D        | +        | -   |
|                                                                        | ZUM3889                       | na        | na          | 2018 | Syngenta  | +       | na                     | 2a-D        | +        | -   |
|                                                                        | P89                           | zucchini  | France      | 2011 | HM.Clause | +       | + on zucchini          | 2a-E        | +        | -   |
|                                                                        | P90                           | zucchini  | USA         | 2011 | HM.Clause | +       | + on zucchini          | 2a-E        | +        | -   |
|                                                                        | Ps pep 66                     | zucchini  | na          | na   | HM.Clause | +       | na                     | 2a-E        | +        | -   |
| Other <i>Pseudomonas syringae</i> strains isolated from cucurbit seeds | 1007009                       | cucumber  | na          | 2010 | VMK       | -       | + on cucumber*         | 2b          | +        | -   |
|                                                                        | 1008020                       | cucumber  | na          | 2010 | VMK       | -       | + on cucumber*         | 2b          | +        | -   |
|                                                                        | PAS3284                       | zucchini  | na          | 2021 | GEVES     | -       | + on cucumber          | 2b          | +        | -   |
|                                                                        | P108                          | zucchini  | Chile       | 2011 | HM.Clause | -       | + on zucchini          | 2b          | +        | -   |
|                                                                        | P135                          | zucchini  | India       | 2014 | HM.Clause | -       | + on zucchini          | 2d          | +        | -   |
|                                                                        | CFBP6576                      | melon     | France      | 1992 | CFBP      | -       | + on zucchini          | 2d          | +        | -   |
|                                                                        | PAS3754                       | pumpkin   | na          | 2023 | GEVES     | -       | + on cucumber          | 2b          | -        | -   |
|                                                                        | PAS3795                       | squash    | na          | 2023 | GEVES     | -       | + on cucumber          | 2b          | -        | -   |
|                                                                        | CI                            | cucumber  | na          | na   | VMK       | -       | + on cucumber*         | 2b          | -        | -   |
|                                                                        | CFBP6452                      | melon     | New-Zealand | 1977 | CFBP      | -       | + on zucchini          | 2b          | -        | -   |
| Non-target strains                                                     | PAS3618                       | pumpkin   | na          | 2022 | GEVES     | -       | - on cucumber          | 2b          | -        | -   |
|                                                                        | PAS3619                       | pumpkin   | na          | 2022 | GEVES     | -       | - on cucumber          | 2b          | -        | -   |
|                                                                        | PAS3620                       | pumpkin   | na          | 2022 | GEVES     | -       | - on cucumber          | 2b          | -        | -   |
|                                                                        | <i>Xanthomonas cucurbitae</i> | zucchini  | na          | na   | HM.Clause | -       | + <i>X. cucurbitae</i> | na          | -        | -   |
|                                                                        | <i>Acidovorax citrulli</i>    | na        | na          | na   | na        | na      | + <i>A. citrulli</i>   | na          | -        | -   |
|                                                                        | PAS156                        | cabbage   | na          | na   | GEVES     | -       | + <i>maculicola</i>    | 1a          | -        | +   |
|                                                                        | PAS3794                       | radish    | na          | 2023 | GEVES     | -       | + <i>maculicola</i>    | 1a          | -        | -   |
|                                                                        | Psm280314                     | radish    | na          | 2014 | VMK       | -       | + <i>maculicola</i>    | na          | -        | -   |
|                                                                        | CFBP1617                      | beet      | USA         | 1959 | CFBP      | -       | + <i>aptata</i>        | 2b          | +        | -   |
|                                                                        | PAS3781                       | beet      | na          | 2023 | GEVES     | -       | + <i>aptata</i>        | 2b          | +        | -   |
| Non-target strains                                                     | PAS2668                       | bean      | na          | 2020 | GEVES     | -       | + <i>Pss</i>           | 2d          | +        | -   |
|                                                                        | CFBP4888                      | bean      | USA         | 1999 | CFBP      | -       | + <i>Pss</i>           | 2d          | +        | -   |
|                                                                        | <i>Pseudomonas corrugata</i>  | tomato    | France      | 1986 | CFBP      | -       | + <i>corrugata</i>     | na          | -        | -   |
|                                                                        | PAS3750                       | parsley   | na          | 2023 | GEVES     | -       | + <i>apii</i>          | na          | -        | -   |
|                                                                        | PAS3574                       | coriander | na          | 2022 | GEVES     | -       | + <i>coriandricola</i> | na          | -        | -   |
|                                                                        | CFBP2067                      | sunflower | Mexico      | 1999 | CFBP      | -       | + <i>helianthi</i>     | 6           | -        | -   |
|                                                                        | CFBP6471                      | bean      | France      | 1999 | GEVES     | -       | + <i>Psph</i>          | na          | -        | -   |
|                                                                        | CFBP2107                      | bean      | Switzerland | 1927 | CFBP      | -       | + <i>viridiflava</i>   | na          | -        | -   |
|                                                                        | CFBP6472                      | pea       | France      | 1997 | CFBP      | -       | + <i>pisi</i>          | na          | -        | -   |
|                                                                        | CFBP6470                      | soybean   | France      | 1994 | CFBP      | -       | + <i>glycinea</i>      | na          | -        | -   |
| Non-target strains                                                     | CFBP1427                      | tomato    | France      | 1972 | CFBP      | -       | + <i>tomato</i>        | na          | -        | -   |
|                                                                        | CFBP2101                      | chicory   | na          | 1929 | CFBP      | -       | na                     | na          | -        | -   |
|                                                                        | CFBP8589                      | tobacco   | USA         | 1905 | CFBP      | -       | na                     | na          | -        | -   |

### 3. VCZ specificity and sensitivity:

- 4Ba primers/probe** (Lacault et al., 2023)
- Amplification of **24 out of 24 VCZ strains**
- Amplification of **8 strains out of 12 of *P. syringae* pathogenic**
- Amplification of **3 strains out of 4 of '*aptata*'** and **2 out of 2 *Pss* strains** (previously reported in Lacault et al., 2023)
- No-amplification of the **4 remaining *P. syringae* pathogenic**, the **3 *P. syringae* non-pathogenic**, the **5 *P. syringae* strains** and the **28 other non-target**

Addition of *P. syringae* strains isolated from cucurbit seeds to the strain collection  
**non-pathogenic = non-target**  
**pathogenic = target** (with VCZ primers/probe)

|            | Expected +  | Expected -      |
|------------|-------------|-----------------|
| Obtained + | 24 + 8 = 32 | 3 + 2 = 5       |
| Obtained - | 4           | 3 + 5 + 28 = 36 |

Specificity = **87.80 %**

Sensitivity = **88.89 %**

### 4. Repeatability and Reproducibility of qPCR:

- 9 target strains:**
  - 2 ALS**
  - 5 VCZ**
  - 2 *P. syringae* patho.**
- 6 non-target strains:**
  - 1 *P. syringae* non-patho.**
  - 5 other non-target**
- Repeatability:** test of 3 replicates of each strain under the same experimental conditions (same operator, date, equipment, etc.)
- Reproducibility:** test of 3 replicates of each strain under different experimental conditions (different operators, dates, equipment, etc.)

Repeatability = **100 %**

Reproducibility = **100 %**

## Conclusions

### ➤ ALS strains:

- Excellent specificity and sensitivity**
- P. syringae* pv. *lachrymans* often poorly identified and therefore rare → **more strains** would be ideal to confirm results
- Detection of *P. syringae* pv. *maculicola* → **not pathogenic on cucurbits** but on brassicas
- Pathogenicity assay** to identify ALS strains **no longer required**

### ➤ VCZ strains:

- Good specificity and sensitivity**
- Detection of *Pss* and *P. syringae* pv. *aptata* → **respectively not pathogenic on cucurbits and not cucurbits seed-borne**
- Detection of *P. syringae* pathogenic → **addition to target strains**
- Improving the sensitivity** of the qPCR method to identify all *P. syringae* pathogenic strains

### ➤ Prospects:

- New service** available at GEVES: Isolation on medium and **identification of ALS, VCZ and *P. syringae* pathogenic** strains by **multiplex qPCR**
- Save time** (at least 14 days) and **reduced costs**
- Confirmation by **pathogenicity assay** as an option
- Development of a detection method directly from cucurbit seed macerates by **SE-qPCR**
- Potential **ISF ISHI-Veg** project to come

### ➤ Acknowledgements:

- GEVES staff** who isolated strains from the collection and participated in the validation of this method.
- The National Seed Health System (NSHS), the French Collection for Plant Associated Bacteria (CFBP), Syngenta and Vilmorin-Mikado (VMK) for supplying strains from the collection.
- HM.Clause (HMC) for supplying strains from the collection and for its expertise on the VCZ strains.