Implementation of the detection protocol for *Xanthomonas euvesicatoria* in pepper seeds

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The disease

- Bacterial spot is a worldwide disease, mainly affecting tomato and pepper.
- Symptoms may affect all aerial parts and are particularly severe on pepper.
The causal agent

- The causal agent was formerly known as *Xanthomonas campestris* pv. *vesicatoria*.
  - Now reclassified into four species:
    - *Xanthomonas vesicatoria*
    - *Xanthomonas euvesicatoria*
    - *Xanthomonas perforans*
    - *Xanthomonas gardneri*
- All four species are regulated
The EPPO diagnostic protocol

Flow diagram for testing tomato and pepper seeds to detect *Xanthomonas* spp.
Objective or our studies

- Implement the diagnostic protocol for pepper seeds.
  - In particular, focusing on *X. euvesicatoria*
- Compare two different DNA extraction methods.
- Compare ELISA diagnostic results with PCR and Direct Isolation on agar media.
- Suggest implementation of the DP
Material and Methods

- During the analytical season 2014 on pepper seeds in Novi Sad (Serbia), 13 seeds lots found *Xeuv* positive
  - Test applied: DAS-ELISA (Loewe)
- The second lab sample was taken for:
  - Direct isolation on YGCA
  - Preparation of seed extracts for PCR
    - Heat shock
    - DNeasy Plant Mini Kit columns (Qiagen)
Material and Methods

- PCR protocol applied on seed extracts:
  - EPPO
    - Primer pairs (Koenraadt et al., 2007)
- For the identification of putative *Xeuv* colonies obtained on YGCA:
  - BOX, REP, ERIC
- Analysis of genetic profiles:
  - UPMGA Cluster analysis (GelCompar 4.1, Applied Maths, Kortrijk, Belgium)
  - Pearson’s correlation coefficient
## Results

<table>
<thead>
<tr>
<th>Samples (varieties or breeding lines)</th>
<th>ELISA</th>
<th>Direct isolation, confirmed by PCR on pure cultures and genotyping</th>
<th>PCR DNA extraction with heating shock</th>
<th>PCR DNA extraction with DNeasy Plant Mini Kit</th>
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Results – ERIC PCR
Results - Clustering
Discussion and Comments

- Comparing results:
  - PCR is best done by previous DNA extraction and purification using mini columns
  - PCR confirmed most ELISA results
  - Direct isolation is not so sensitive

- Identifying *Xeuv*
  - Genotyping not always able to discriminate *Xeuv* from *Xper*
  - REP primers are more discriminative than BOX or ERIC
Thank you for your attention!