DNA technologies to combat seed pathogens

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Combat seed pathogens

• Food safety
  • staple crops

• Environment
  • escape of pathogens into native crops/flora

• Economic
  • financial losses of seed companies and farmers/growers
Combat seed pathogens

- Seed borne
  - Not necessarily transmission to plant

- Seed transmitted
  - Type of pathogen
  - Environmental conditions
Advantages of DNA technologies

• Universal technology
• PCR
  • sensitive detection
  • specific identification
• Next Generation Sequencing
  • increasing amount of information
  • cost effective
Applications

• Disease testing
  • detection and identification of (seed) pathogens
  • diagnostics

• Identification
  • plant varieties
  • bacteria / fungi
Applications

- Disease testing
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Applications

Diagnostics
the process of attempting to identify a possible disease or disorder (symptomatic material)

Routine testing
a test or group of tests performed to screen for the absence (or presence) of specific pathogens (that may be present latently)
PCR to detect seed pathogens

• PRO
  • universal
  • DNA & RNA
  • detection and identification
  • relatively fast implementation
  • constant quality of primers and probes production
  • test results quickly available

• PRO & CONTRA
  • sensitive technology
  • specific

• CONTRA
  • seed-remains inhibit PCR
  • still little (validated) sequence information
PCR to detect seed pathogens

**Identification**
- Extract seed
- Plating
- Visual identification of suspect bacteria
- Confirmation by PCR

**Detection**
- Extract seed
- Isolate DNA
- PCR
PCR for seed pathogens

• Identification/confirmation
  • 7-019a: Detection of *Xanthomonas campestris* pv. *campestris* on *Brassica* spp.
  • 7-020: Detection of *Xanthomonas hortorum* pv. *carotae* on *Daucus carota* (carrot)
  • 7-030: Detection of *Acidovorax valerianellae* on *Valerianella locusta* (corn salad)

• Detection
  • detection of pospiviroids in tomato/pepper seed (ISHI/Naktuinbouw)
  • direct PCR for detecting *Acidovorax citrulli* (TESTA)
  • direct PCR for detection of *Xanthomonas campestris* pv. *campestris* & *Pseudomonas syringae* pv. *maculicola* (TESTA)
PCR identification/confirmation

• To confirm identity of pathogen
  • direct on isolate
  • specific primer sets required (specificity)
    • preferably use more primer sets
  • sensitivity less relevant
PCR detection

- Detect pathogen directly in seed extract
  - low level of pathogens (sensitivity)
  - seed matrix inhibits PCR (sensitivity)
  - specific primers required (specificity)
  - use of spike

- Spike
  - add a comparable pathogen to the extract to determine recovery
    - control on extraction
    - control on PCR
PCR detection

• Detect pathogen directly in seed extract
  • low level of pathogens (sensitivity)
  • seed matrix inhibits PCR (sensitivity)
  • specific primers required (specificity)
  • use of spike

• No isolate in hand
  • alternative conformation desirable
PCR detection: A. *citrulli*

- is causal organism of bacterial fruit blotch (BFB)
- Several hosts amongst the cucurbits
- Seed transmittable

Detection methods
  - Grow-out (greenhouse or sweat box)
  - Direct seed wash PCR
PCR detection: *A. citrulli*

- Extract of 5000 seeds
- Add low number of *A. cattleyae* as spike
- Purify DNA with DNA extraction kit
- Perform multiplex Taqman PCR
PCR detection: *A. citrulli*

- Sensitivity
  - < 10 cells *A. citrulli* /mL seed extract

- Good specificity

- Repeatability and reproducibility
  - 100%

Video available: www.seedtesta.eu
Applications

- Disease testing
  - detection and identification of (seed) pathogens
  - diagnostics

- Identification
  - plant varieties
  - bacteria / fungi
# Identification of (known) pathogens


<table>
<thead>
<tr>
<th>Identification Level</th>
<th>Sequencing &amp; Barcoding</th>
<th>PCR</th>
<th>Molecular Markers</th>
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Molecular markers

• Various technologies to identify randomly genetic differences
  • AFLP / SSR /SNP

• Distinguish on strain/subspecies level

• Additional information
  • microbiology
  • pathogenicity
  • ‘look alikes’
Clavibacter in tomato seeds
PCR to distinguish *Fusarium* species

- *Fusarium* species infecting wheat seeds
- Identification done by microbiology
- Some species are difficult to distinguish from some others
  => PCR used to differentiate some species
## PCR to distinguish *Fusarium* species

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### Possible confusion based on:

- X Spores and color
- X Color (without sporulation)
- X Sporulation

- Distinction by PCR
- No tools available
- Some false identifications using molecular tools
Barcoding

- Sequence of one or more specific gene(s) are used as ‘barcode’ to univocally identify an organism as belonging to a particular species
Barcoding of 12 combined genes
Next Generation Sequencing (NGS)

- Cost of sequence data decreases
  - capacity increases
- Increasing amount of genetic sequence data
- How are sequences validated?
  - biological background
  - Pathogenicity
- Q-bank
  - curated information on plant pathogens (incl. sequences)
  - www.q-bank.eu
NGS: Applications

• Identify genes involved in pathogenicity
  • compare pathogenic vs. non-pathogenic organisms
  • identify pathogenicity related genes
  • target for PCR development

• Identify diseases in plants (diagnostics)
  • Sequence plant genome or transcriptome
    • DNA/RNA/sRNA
    • identify genes for pathogens
NGS: diagnostics

- citrus vein enation disease
  - graft-transmissible disease of sour orange (*Citrus aurantium* L.)
  - virus like structures visible
  - reactive with *Barley yellow dwarf virus* antiserum
  - no virus isolated

Vives et al (2013) Phytopathology 102, 1077
NGS diagnostics

• Deep sRNA sequencing
  -> identification of 19 virus-derived small RNAs

• PCR
  -> to construct full genome sequence of 5,983 nt

• Identification of new virus: *Citrus vein enation virus* (CVEV)

Vives et al (2013) Phytopathology 102, 1077
Developments

• Novel DNA/RNA methods
  • high throughput
  • multiplex (e.g. Luminex)
  • on site detection (e.g. LAMP)

• Next Generation Sequencing
  • the new standard

• Alive or dead
Conclusion

• DNA based technologies for reliable detection of (seed) pathogens

• Next Generation Sequencing will offer new opportunities
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