Molecular and Biological Testing Methods: How they work and what they tell us

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Reference Paper from ISF

ISF Viewpoint on Indirect Seed Health Tests

http://www.worldseed.org/cms/medias/file/ PositionPapers/OnSpecificTechnicalSubjects/I ndirect_Seed_Health_Tests_2013.pdf

Background

- Seed Health is often the intersection of quality concerns (for a company) and phytosanitary concerns (for a country)
- For seed companies,
 - Vegetable Seed Consumers EXPECT healthy, disease-free seeds
 - Outbreaks associated with seed borne pathogens can be:
 - Extremely costly
 - Damaging to our reputation
 - Affect reliability of supply
- For countries, they want seeds that will not introduce a pathogen (change country production success)
- Shared Goal: Deliver seeds that enable customers to produce a healthy, vigorous crop (fruits and vegetables)

International Seed Health Initiative

- From the Industry side, there is an established initiative that focuses on the development and validation of seed health methods: ISHI
- Brings seed companies, private laboratories and public sector institutions together to address seed health issues
 - Seed-transmitted diseases that may have an impact on trade and/or crop health
- Currently, 55 active scientists (plant pathologists, molecular biologists)
 NL, US, FR, JP, IL, ES (also KO, TH, PH, CL, IN)
- Represents ~90% of the vegetable seed traded internationally (measured in USD)

Fundamentals of ISHI-Veg

- Seed Health area is considered a NON-COMPETITIVE and non-proprietary
 - Shared data, methods, seed sources, microbial isolates, and experience
 - Shared lessons from SH-related complaints
- All methods are shared on ISF website: <u>http://www.worldseed.org/isf/ishi_vegetable.html</u>
- Methods may also be shared and/or validated and reviewed with other organizations:
 - International Seed Testing Association
 - National Seed Health System of USDA
 - National Plant Protection Organizations

Seed Health Method Types

- Direct Methods (Biological)
 - Result in the recovery and confirmation of a pathogen from a sample
 - Bacterial seed wash with pathogenicity assay
 - Fungal blotter assays (with microscopy)
 - Seedling grow outs
- Indirect Methods (Molecular)
 - Result in a data point that is *correlated* to pathogen presence
 - Bacterial seed wash without pathogenicity assay
 - Direct Seed or Seed-wash PCR
 - ELISA

Observations or Results from Biological and Molecular Methods





Biological

Molecular

Differences in Resources for Method Execution

Drivers	Biological	Molecular
Time (Duration of assay)	Few as 6 days Up to 42 days	1 day-3 days
Resources	Lab equipment; Greenhouse or Growth Chamber space	Lab equipment
Cost	Highly variable (\$50 to \$2000 per sample)	Variable generally more consistent (\$100-200 per sample)
Expertise	High level of expertise on morphology, symptomology (performing and evaluating)	Expertise on GLP (performing)
Conclusions	Pathogen presence confirmed	Pathogen presence assumed

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Methods of the future....

- Companies and Countries are embracing the advantages of molecular testing...
 - Which may also include: Improved Specificity, Improved Repeatability
- New tests may look like this:



• Fixed per assay

Nucleic Acid Extraction

 Variable: Laboratory- or Company-specific methods Analysis

 Fixed per assay (pathogen target)

Why did it take so long to get here?

- PCR has been around for decades
- New methods have a similar flow as compared with trait testing
- Key difference:
 - Seed health involves organisms that are undergoing evolution in real-time (reproductive time is minutes to hours, not years)
 - Genetic mutations → genotypic and phenotypic differences
 - How to ensure detection? Need assays designed based on data from large populations of target pathogen

Genome Sequencing: Public + Monsanto Genomic Data

Xevt-82i contid0003

Xcvt-86i_contig00023

Cevt-10i contia0001

XevT-165i_contig00036

Xcvt-74i_contig00033

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Kort-38, contig00023
 Kort-38, contig00023
 Santhromonas gardneri, strain_LD57, F1-F0-ATPase_subunit
 Xanthromonas gardneri, strain_JS750-JF1-F0-ATPase_subunit
 Xanthromonas gardneri, strain_JS749-JF1-F0-ATPase_subunit
 Xanthromonas gardneri, strain_J0715, F1-F0-ATPase_subunit
 Xanthromonas gardneri, strain_J0715, F1-F0-ATPase_subunit
 Xanthromonas gardneri, strain_J0711, F1-0-ATPase_subunit
 Xanthromonas gardneri, strain_J0711, F1-0-ATPase_subunit
 Xanthromonas gardneri, strain_J0712, F1-0-ATPase_subunit
 Xanthromonas gardneri, strain_J0712, F1-0-ATPase_subunit
 Xanthromonas gardneri, strain_J0712, F1-0-ATPase_subunit
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P. euvesicatoria

P. perforans

P. vesicatoria

P. gardneri

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Xcvt-89i_contig00016 Xcvt-127i_contig00032 XcvT-110i_contig00045 00414

Xcvb-27i_contig00414 72i contig00031

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> XcvP-200i_contig00041 -XcvP-113i_contig00010 -Xcvp-21i_contig00045 -Xcvp-6i_contig00166 -Xcvt-126i_contig00020 Xcvt-68i_contig00055

Xcvt-84i_contig00019 Xanthomonas_euvesicatoria_strain_CFBP3268_F1-F0-ATPase_subunit Xanthomonas_euvesicatoria_strain_CFBP5618_F1-F0-ATPase_subun

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> > Xcvt-48i_contig00041 Xcvt-57i contig00048

> > > Xovt-60i_contig00053

Xcvt-66i_contig00096/ / Xcvt-76i_contig00011/

Xcvt-80i_contig00026

XcvP-152i_contig0005

PCR assay development

- Need broad populations
 - ISHI companies
 - Academic Collaborators
 - Public collections and databases
- Need to have a robust process for evaluating newly designed primers
 - Applied to culture collections
 - Applied to routine seed samples (target and non-target isolates, seed washes, plate washes, etc.)
 - Within ISHI, we select primers based on performance
 - Zero percent false negatives
 - Minimal false positives
 - Constant monitoring of assay performance leads to frequent improvements

Final Thoughts

- Molecular methods have advantages over biological methods in speed, resources, technical expertise
 - BUT they do not CONFIRM pathogen presence
 - At best they INDICATE
- Biological methods are still needed to confirm the indicators of molecular tests
- Preferred approach:
 - Start with molecular methods as a screen
 - If results do not indicate pathogen presence, release seed for use
 - If results indicate a possible detect, follow up with biological method
 - Generally, pathogen detects on seed are not common (less than 1%) therefore most samples can be released quickly

Thanks for your time

Questions???